CLINICAL AND BIOLOGICAL INVESTIGATIONS OF MASTALGIA

ROBERT EDWARD MANSEL

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ABSTRACT

The treatment of breast pain associated with benign breast disease has been largely unsatisfactory due to both the unavailability of effective therapies and the lack of proper evaluation of the treatments that did exist. A detailed series of controlled studies of new agents for breast pain has been carried out using standardised trial methodology and recording proformas. Changes in symptoms have been carefully documented using a variety of methods which have been compared for accuracy and usefulness for general use in surgical clinics. Biopsies from women with mastalgia have been compared with other benign disease groups using monoclonal antibody immunohistochemistry to study the expression of an oestrogen-regulated protein.

Three agents with fundamentally different modes of action have been tested against placebo treatment in carefully defined cyclical and non-cyclical mastalgia. All three agents have been shown to significantly improve breast symptoms compared with placebo , but with varying levels of efficacy and response times. Danazol, an anti-gonadotrophin, had the highest response rate, followed by bromocriptine, a prolactin lowering drug. The least effective treatment was dietary supplementation with evening primrose oil, which also gave the slowest response time.

However, side effects of the three agents were also considerably different in frequency and severity, with evening

primrose oil having virtually no side effects, while bromocriptine had the highest frequency of side effects. Laboratory investigations of serum hormones and fatty acids have indicated different mechanisms of action of the 3 agents. Study of the p24 oestrogen-regulated protein content in breast tissue showed no evidence of enhanced oestrogen activity in mastalgia patients.

These studies have introduced new guidelines for treatment for women with painful nodular breasts, and were the first large properly controlled trials of these 3 drugs to be performed for mastalgia.

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DECLARATION

I hereby declare that the studies described in this thesis are the result of my own design and work, except where reference has been made to assistance by my research fellows. The material herein has never been previously presented, either wholly or in part, for any degree and is not being concurrently submitted for any other degree.

R.E.Mansel

Candidate

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DEDICATION

To my father, Regnier

Pleasure reaches its limits in the removal of all pain

EPICURUS, Sovran Maxims.No3

INTRODUCTION

Pain in the breast is the commonest reason for breast related consultation in both general practice and the surgical clinic (Nichols et al 1980). Yet, despite its frequency, little attention is paid to the subject, in general or specialist texts devoted to breast or gynaecological diseases. The underlying reasons for this are the generally poor understanding of the aetiology of the condition, and the overwhelming attention devoted to breast cancer by clinician and research worker alike.

The studies described in this thesis were initiated as a direct result of the paucity of knowledge on the aetiology and management of the symptoms of pain and nodularity in patients with benign breast disease. The only honest answer to the question from a patient, "Why do I have painful breasts and what can be done about them ?", is an admission that the cause is unknown and the current body of knowledge has virtually nothing of practical value on treatment. Although the subject has attracted the interest of eminent physicians over many centuries, little has emerged that is of direct benefit to the patients. Further, the interest of the average physician has focused inexorably on the significance of pain in the differential diagnosis of breast cancer, while ignoring the significance, both mental and physical, of the pain to the bearer of the painful breast. In reality, breast pain has little or no diagnostic value in breast cancer, (excepting locally advanced cancer), as there are no specific features that will distinguish pain associated

with early breast cancer from the benign causes of pain. The aim of these studies has been to examine breast pain without the connection with breast cancer, and to study new therapeutic agents which have become available in recent years.

As will be seen from the historical review, the variety and empiricism of previous treatments merely confirms their uselessness, yet recognises , at least in the view of some physicians, that treatment for the symptom of breast pain was indicated. Some physicians feel that treatment with active drugs is unnecessary, although most would admit that there is a small subgroup of patients who suffer sufficient breast pain to consider treatment. These patients with severe symptoms are the subjects of the studies described herein, and the rationale for their treatment is simply the alleviation of pain and associated symptoms. Since, by definition, the complaints are subjective and cannot be measured in any objective manner, the existence of the problem of breast pain has to depend on the reporting of the individual patient. The severity of the problem in some patients may be inferred from the repeated visits made to general practioners and hospital out-patients departments over several years, in search of relief from this non-life-threatening condition. These studies, and others over many years, have convinced the author that treatment is indicated in some patients, and should be offered to those patients who find their breast pain interferes with everyday activities. It behoves those physicians who contend that treatment for breast pain is unnecessary, to prove that their patients are not suffering

pain. The cursory dismissal of these patients by some physicians is due to the scanty and inadequate literature on this subject, as well as the universal tendency among physicians to ignore conditions they do not understand, or, even worse, label the patients with that ill-defined and all embracing term "neurotic". A classic example of this behaviour was recently reported by a doctor who developed pain in the pelvic area, and was labelled "neurotic" by some of his colleagues, because repeated investigations failed to show a physical cause for the pain (Kessel 1977). What was more remarkable, was the fact that the doctor was a consultant in orthopaedic surgery and was convinced his pain was "organic". The mystery was solved when the newly discovered condition of cauda equina syndrome was described, and proved to be the cause of his symptoms. This personal article makes interesting reading and illustrates clearly the scepticism of physicians when they encounter a condition they do not understand.

The older treatments had little or no rational basis for their use because the pathophysiology of benign breast disease was not understood. It was quickly discovered that some ovarian factor was implicated, but ignorance of the normal workings of the breast remained profound, while knowledge of disordered physiology was non-existent. While it must be admitted that the precise mechanisms involved in the pathophysiology of human benign breast disease are still not clearly understood, there have been several new findings which have shed some light on the matter. The main stimuli to this new knowledge have been

the rapid development of methodology such as radioimmunoassay (RIA), new immunohistochemical techniques over the last decade, and the intense study of the epidemiology of benign breast disease, reflecting recent interest in hormonally related risk factors in breast cancer. Detailed studies of the pituitary-ovarian axis using radioimmunoassay have shown differences between some mastalgia subgroups and normals in both basal levels of ovarian hormones and stimulated levels of pituitary hormones. While these studies do not clearly identify a single common pathophysiological mechanism for painful benign breast disease, they allow the identification of a particular segment of the complex hormonal cascade, which can then be manipulated with a semblance of rationale. Hand in hand with the new knowledge of hormone physiology has come the means of producing subtle yet precise alterations of hormones at many different levels in the pituitary-ovarian axis. The development of several new and often unique drugs has allowed single hormones to be altered by the investigator, and has increased knowledge of the roles of the individual hormones in those complex interactive schemes that form the basis of modern reproductive endocrinology.

The studies described in this thesis were carried out between 1976 and 1984, and formed a detailed and continuing series of studies of the treatment and biological basis of mastalgia. The classification and background of breast pain was first studied by Mr Paul Preece FRCS and written up in his MD thesis of 1982 (Preece 1982). The drugs to be tested in mastalgia

were selected empirically, but a controlled trial format was used for each. I designed the concept, trial structure, methodology, and assessment of response for all the trials reported here, and created all the pain charts, visual linear analogue and trial forms shown in this thesis. I saw every patient in the bromocriptine trial from entry to completion, and saw every patient in the danazol trial on entry. The assessment of the patients in the danazol trial was jointly done by Dr J. Wisbey and myself, while the assessments in the evening primrose oil trials were carried out by myself, Dr N. Pashby and Mr J.K. Pye. All were research fellows working directly under my instructions and using trial designs and paperwork developed from my study on bromocriptine and danazol.

I performed all of the immunohistochemical techniques described in chapter five, after spending 1 year in laboratory research with Professor W. McGuire at the University of Texas in San Antonio. All of the tissue specimens described in Chapter five were stained by myself or Mr S. Courtney whom I taught to perform the technique on my return from the U.S.A.

The studies described herein attempt to construct a more rational approach to the treatment of breast pain based on new pathophysiological findings, and aim to define the roles of the new drugs in mastalgia treatment . The selection of patients for treatment was based on a symptomatic classification which is routinely used at the Breast Clinic in the University Hospital of Wales in Cardiff. The effects of each drug were tested in

controlled trials designed to minimise the effects of confounding variables, especially the placebo effect. The studies have demonstrated the efficacy of the drugs studied, and have defined certain patient characteristics which will allow better selection for treatment. These studies will give practical guidance to physicians who wish to treat patients with breast pain but find patient and drug selection confusing. Further, they will increase the probability of a first drug response and thus save side effects for the patient and money for the Health Service. These studies should allow patients with severe mastalgia to be prescribed treatments based on efficacy and side effects rather than empiricism.

CHAPTER ONE

HISTORICAL REVIEW OF THE TREATMENT OF MASTALGIA

As hormones were unknown until relatively recently, little was written on the treatment of breast pain and nodularity until early this century. Several accounts of the clinical picture and natural history of painful benign breast disease had been written in the 19th century often with accurate descriptions, but the lack of any identifiable cause led to the adoption of highly empirical and sometimes bizarre treatments. Many authors concluded that women who complained of breast pain were "neurotic", but this was not tested formally, and depended more upon the physician's frustration with a condition he could not understand, rather than the patient's psyche. One of the earliest contributors to the literature was the famous surgeon Astley Cooper from St.Bartholomew's Hospital, who accurately described mastalgia in 1829, (using the term neuralgia), and pointed out the common association with nodularity (Cooper 1829). However, he dismissed these women with mastalgia as being

> "of an irritable and nervous temperament, with excessive excitability of the system".

He was referring, presumably, to the excitability of their higher central nervous system, although it might be suggested charitably that he was ahead of his time, and was really predicting recent work showing increased pituitary excitability in mastalgia published over 150 years later (Kumar et al 1984).

Birkett in 1850 published a book on "The diseases of the breast and their treatment" (Birkett 1850), in which he gave a good description of breast pain and nodularity, but despite the

title of the book, wrote little of practical value on treatment. Many of these early authors concentrated on the importance of mastalgia in the differential diagnosis of breast cancer, but it was known that most breast cancers were painless, unless local musculo-skeletal infiltration was advanced (Velpeau 1856, Billroth 1879, Bryant 1887). However, Billroth's contribution was particularly important as he noted that different types of mastalgia occur and that the evidence for the various treatments was lacking. Further, he suggested that an important part of therapy was firm reassurance that the condition was benign , an observation which still holds good today.

Over the next 40 years several reports of individual cases of breast pain were reported, again with widely differing treatments being recommended, mostly based on empirical remedies. This era also saw the introduction of the term "chronic mastitis", which still causes so much confusion today. Despite the widely differing terminology, the pattern of cyclical mastalgia presentation can often be identified in these reports, suggesting that the presentation has not changed very much. Therapy in the early years of this century continued to be directed at the supposed aetiologies of mastalgia and lumpiness. Cheatle suggested trauma from clothing as a cause of mastitis (Cheatle 1911), somewhat mimicking the modern suggestion that ill-fitting brassieres are the cause of mastalgia (Wilson and Sellwood 1976). Keynes suggested in his Hunterian oration that chemical irritation secondary to stagnating secretions was implicated in mastitis (Keynes 1923). He advocated either

amputation of the breast or radiation therapy for severe cases of breast pain, and suggested that marriage and pregnancy would help the less severe cyclical pain in young women (Keynes 1923).

Around this period, breast pain, unrelated to the menstrual cycle (non-cyclical mastalgia), was being recognised. This was described as a chronic pain localised to the costal cartilages by Tietze (Tietze 1921), and conservative treatment was advocated. An earlier description of what was probably the syndrome named after Tietze was described in a letter to the Lancet in 1914 (WHC 1914).

A gradual realisation of the importance of the ovary and the other endocrine organs in lactation and breast growth, began to direct attention to the role of internal secretions, as yet undefined by the term "hormones". The classical report of Beatson (Beatson 1896) describing regression of breast cancer after oophorectomy, demonstrated a definite link between the ovary and the breast. Hastrup reported the cure of severe mastalgia by correction of uterine retroversion and removal of an enlarged ovary, although this may have been an example of a hormone producing tumour (Hastrup 1911). Increasing use was made of extracts of gonadal tissue, and reports of the use of corpus luteum extract (Lisser 1918, Samuel 1928), testosterone (Tailhefer 1938, Desmarest 1938, Spence 1939, Atkins 1940) and also bilateral oophorectomy (Leriche 1923) were published. Since knowledge of the existence and function of hormones was scanty, there were inevitable conflicts in the explanations offered for

the various ovarian manipulations reported. Some believed that corpus luteum function was increased (Cutler 1931, Bertner 1932) while others believed that the output of the corpus luteum was too low (Geschickter 1941, Pollosson et al 1951). Much of the emerging knowledge of hormones was coming from animal work, and even in 1936 it was suggested that oestrin (oestrogen) causes ductal proliferation, while corpeus luteum hormone (progesterone) in combination with oestrogen was responsible for acinar growth (Nelson 1936). Even at this stage the possible link between epithelial proliferation induced by oestrogen and induction of breast cancer was being raised (Lacassagne 1936), but, as David Patey wisely pointed out (Patey 1938), direct extrapolation from rodent to human physiology may be unsafe, and lack of awareness of this fact has led to a great deal of confusing work in the last 40 years when unwarranted implications for human disease have been made on the basis of pure rodent work. Patey described mastalgia and cystic disease in 1949 and clearly distinguished between them on clinical grounds; he further stated that neither condition was related to subsequent carcinoma (Patey 1949). Two years later he succinctly described the problems of nomenclature, especially the use of the term "chronic mastitis", which he felt had no meaning (Patey 1951). A quotation from the latter paper summarised the problem of the term "chronic mastitis":

"The answer would seem to be that the term chronic mastitis is the expression of an attempt to define in terms of organic disease a symptom - pain in the breast".

Despite these problems, many of these workers set out

to examine carefully the role of hormonal therapy in humans, and the pioneering clinics run by Spence and Atkins in London were specifically set up to study the problem of symptomatic benign breast disease. Spence studied the use of testosterone in a systematic way and used both injection and local application to the breasts (Spence 1939, 1940), based on the idea that the aetiology of chronic mastitis was an excessive output of oestrogen by the ovaries, an idea which still survives today. He showed that about 75% of the patients treated by testosterone had dramatic improvement in their symptoms whether treatment was by injection or local application. Of special note is his attempt to control the subjective element in his assessments by initially giving an identical placebo cream in the group treated by local application (Spence 1940) - he showed a 25% placebo response, very similar to modern studies - another lesson that has been ignored by a large number of clinicians who still carry out studies in breast pain without proper controls or placebo groups. Spence stated in his 1939 paper that:

"the reports of observers on the relief of pain in chronic mastitis with hormone preparations are valueless without proper control" (Spence 1939).

Atkins (fig 1.1) also set out with an endocrinologist, Dr Bishop, to examine patients with chronic mastitis, and in just over 2 years they reported on 212 patients with breast pain and/or nodularity, a fact which suggests that mastalgia and nodularity were common in the 1930's. Atkins defined chronic mastitis as

"pain or lumpiness of the breast not due to bacterial inflammation, new growth or fat necrosis",

which he fully described in his Hunterian Lecture in 1938 (Atkins 1938) and thus made no pre-judgement on the aetiology of the condition; another good reason for the modern use of the term "mastalgia". Atkins' work is important as it laid down important principles that are relevant today (Atkins 1940). He noted that there was no clear cut line between normal and abnormal symptoms, the criterion for treatment being the patient's appreciation of the severity of her symptoms. He further showed that there was no correlation between symptoms and the histopathological picture in the breast - a fact which is all too often disregarded in modern papers discussing the subsequent cancer risk of benign breast disease. He showed that testosterone by injection gave an improvement in pain and nodularity in 75% of patients, whereas oestrogen produced improvement in only 12%. These studies were notable as they were controlled by the use of a preliminary placebo administration. A further important point is that of the 212 patients who had attended the clinic, only 30% were reported to have been treated, and although this is higher than the current Cardiff Mastalgia Clinic figure of 8% of all new breast cases (which include cancers), it did make the point that only a minority of patients seeking advice actually required active drug therapy. The only discordant note in this seminal work was the contention that the women are "apprehensive and mentally unstable", but this statement was based on opinion and no formal assessment of psyche was made at this enlightened clinic. He published further papers on this subject, but the

later papers concentrated on nomenclature and no new data on the treatment of mastalgia appeared (Atkins 1947, 1949, 1951). In his last paper on breast pain, he speculated that the mastalgia seen in premenopausal women was due to distortion and pressure of nerve endings produced by proliferating fibroblasts (Atkins 1952). Nevertheless, his pioneering work represents a serious attempt to establish a scientific foundation for the assessment of therapy of breast pain and nodularity.

Further advances in endocrinology prompted the investigation of the role of the pituitary in breast disease, and several authors suggested therapy based on possible manipulation of pituitary secretion (Gabrielianz 1933, Taylor 1942, Hesser 1944). Taylor's detailed studies of chronic mastitis from the Memorial Hospital, New York, described various hormonal therapies in 68 patients, but he found only temporary improvement on androgens (Taylor 1942). He did note that spontaneous resolution occurred in 50% of the patients who were simply followed up for 2 years. Morton used injections of chorionic gonadotropin in attempts to increase corpus luteum output and reported improvement in both symptoms and histology in 3 patients (Morton 1946).

A thorough review of the problem of breast pain was written by Geschickter, in America, in his book on breast diseases in which he described the clinical features, natural history and treatment of painful nodular breasts (Geschickter 1945). He noted the varying presentations of mastalgia but used

the term mastodynia, which he defined as persistent premenstrual pain felt in an area of thickening in the upper outer quadrant of the breast. He described a series of 375 cases in detail and noted a mean age of 35 years with an average duration of pain of 21.5 months, features which fit closely with the cyclical mastalgia pattern described in Cardiff. On the basis of a small number of urinary hormone estimations he concluded that an absolute or relative hyperoestrogenism was the cause of mastodynia, and stated that progesterone was a logical treatment. Despite this statement, 93 patients were treated by surgical excisions (29 mastectomies) and only 35 patients were treated by endocrine therapy. He initially used estrone injection in 19 patients with good results in 16,and then progesterone injections in 15 patients with good results in 13. No controls were used and the criteria of response were not stated. He used testosterone on a few patients with good results.

Androgens continued to be the mainstay of therapy from around 1945 until new progestogens and androgen derivatives began to appear in the early 1960's when steroid chemists began to alter the configurations of the steroid skeleton. In 1961 Reerink et al announced a new class of active steroids which they named retrosteroids (Reerink et al 1961) where the C9 hydrogen was in the B position and the C10 methyl group was in the A position. A prominent member of this class, dydrogesterone, was soon shown to be very active progestationally (Bishop 1961) and was used in several pilot studies for the treatment of menstrual cycle abnormalities and the premenstrual tension syndrome (Bishop 1961,

Day 1979). Bishop's study was uncontrolled and showed an 82% response rate for dysmenorrhoea (Bishop 1961), and although the study reported by Day was a placebo controlled study of premenstrual tension, no improvement of breast symptoms was seen on therapy (Day 1979). Various other progestogens, either alone or in combination with oestrogens, have been used and have been reported as having given good results. Enovid (norethynodrel with mestranol) was reported to have given a > 50% relief of symptoms in 65% of 110 women, but at varying dosage and with no control arm (Ariel 1973). Workers in France have concentrated on progesterone or progestogen therapy to correct the postulated luteal deficiency in patients with symptomatic benign breast disease (Sitruk-Ware et al 1977), and have used lynestrenol orally in combination with a progesterone-containing cream (Progestogel) which is applied to the breasts (D'Argent 1974, Mauvais-Jarvis et al 1979) with a claimed 96% resolution of pain and an 85% reduction in nodularity, but a lesser response in cysts and fibrodenomata. The problem with the therapeutic studies emanating from this group (Mauvais-Jarvis et al 1978, Sitruk-Ware et al 1979, Kuttenn et al 1983), is that no placebo controls have been employed, and little detail is available on the criteria of response and selection of patients. Further, the postulated luteal deficiency in BBD has been explored carefully in a series of recent studies using daily assessments of serum progesterone (Swain et al 1973, England et al 1975, Walsh et al 1984b) and salivary progesterone (Read et al 1985 , Kumar et al 1986), but no differences could be detected between controls and groups of carefully defined breast disease and mastalgia. Thus the

aetiological basis of progestogen therapy is not yet substantiated, and there is little evidence from controlled studies to show its efficacy. A double blind trial of lynestrenol in patients with mastodynia carried out in Belgium reported that there were significant improvements on lynestrenol, but detailed study of the results shows that this improvement was solely due to thermographic changes, the reading of which are known to be highly subjective and of uncertain significance (Colin et al 1978). An uncontrolled Italian study using medroxyprogesterone acetate 10mg/day also failed to show objective improvement although the patients felt better subjectively (Onnis et al 1984). A controlled study of 44 patients with "hormonal mastopathy" carried out at the Rachel Forster Hospital in Sydney, showed both subjective and objective improvement when patients took norethisterone compared with placebo treatment (Cuningham and Lang 1962). The methods of assessment were not detailed in this study, but the overall response rate was quoted as 90%, after 12-18 months treatment. Evidence showing the ineffectiveness of progestogens in breast pain comes from studies of the pre-menstrual syndrome, where mastalgia can be a prominent symptom. In two double blind studies, one of dydrogesterone (Day 1979) and one of intramuscular progesterone (Taylor 1977), there was no evidence of improvement in breast symptoms compared with placebo treatment, although there were significant improvements in the other symptoms of the syndrome. A further large double blind study of progesterone given parenterally at doses of 200 and 400mg daily in 39 patients with premenstrual syndrome, showed that progesterone therapy was not better than placebo (Sampson

1979). The current position is that the efficacy of progestogens for the treatment of breast pain has not been proven.

New developments in steroid chemistry were also being applied to the androgens in order to preserve their efficacy in treatment (well documented by Atkins (Atkins 1940)), while reducing the distressing androgenic side effects such as acne and hirsutism. In 1971, Greenblatt reported the first clinical studies of a new synthetic steroid derived from 17a-ethinyl testosterone, which acted as an anti-gonadotrophin in primates (Greenblatt et al 1971). This compound, danazol, was described as an impeded androgen, as it only displayed weak androgenicity even at high dosage, and appeared to be devoid of oestrogenic or progestogenic effects. The endocrinological profile of this drug suggested that it might be useful for the treatment of endometriosis and benign breast disease. An open study from the Greenblatt group reported a 75% response rate of breast pain in patients with fibrocystic disease, but again no control figures were available and the criteria of response were not clearly defined (Asch and Greenblatt 1977). Further reports from these authors of sequentially studied patients appeared but no controls were ever used (Nezhat et al 1980, Greenblatt et al 1980, Greenblatt and Ben-Nun 1980). A further open study of 30 patients from the USA reported that 7 women dropped out due to side effects, but the remaining 23 had complete or partial response to danazol in 3 different doses ranging from 50-200mgs (Baker and Snedecor 1979). The lack of controlled studies in the evaluation of this new drug was typical of most of the therapeutic studies

carried out in mastalgia, and the need for a carefully controlled study of this agent and all the other putative agents in mastalgia was clear.

In parallel with the developments of this unique and specific anti-gonadotrophin, a chance finding by a scientist working with ergot alkaloids led to the discovery of a unique prolactin-suppressing drug called bromocriptine (Fluckiger 1972). This drug profoundly suppressed serum prolactin levels in all species and it was immediately suggested that as prolactin was the primary lactogenic hormone , this new anti-prolactin agent might be useful in the suppression of lactation and in breast disease. The discovery of this drug in 1971 was all the more remarkable as it pre-dated the development of a radio-immunoassay for human prolactin, and its effects on prolactin secretion could only be measured by its profound suppression of human galactorrhoea (Lutterbeck et al 1971) and suppression of lactation (Varga et al 1972). Confirmation that the mechanism of action of this drug was by prolactin suppression was made 1 year later when a specific radioimmunoassay for human prolactin had been developed (Besser et al 1972). Although several other actions of bromocriptine have been discovered subsequently, the predominant action in humans is that of prolactin suppression (Thorner et al 1980). The first study using bromocriptine for breast pain was reported a mere 3 years after the original publication, but this was a small open study of only 15 patients with mastodynia (Schulz et al 1975), which reported an overall 85% response rate. However, the method of assessment

of response was not stated and although reference to a placebo was made, the trial structure was not described. The following year, an even smaller study of only 7 patients was reported but suffered from the same methodological deficiences as the Schulz study (Martin-Comin et al 1976). A controlled study in women with the premenstrual tension syndrome (PMT) did show that bromocriptine produced a statistically significant improvement in breast pain and other PMT symptoms (Benedek-Jaszmann and Hearn-Sturtevant 1976). In view of the shortcomings of the 2 mastalgia studies and the obvious promise of bromocriptine as an endocrine agent shown in the PMT study, it was clear that it would be useful to carry out a large controlled study of this drug in the treatment of breast pain.

Many other therapies have been used for the treatment of breast pain, the exact substance being tried depending on the physician's view of the cause of the "disease". Diuretics are still popular among general practitioners although there is little evidence for their efficacy. It was widely held that breast pain was due to fluid retention caused by vascular engorgement (Swan 1965) and diuretics were recommended in the premenstrual phase of the cycle (Israel 1967, Lang et al 1972, Winterton 1969). Although weight gain and breast swelling have been shown to occur in the premenstrual phase of the cycle (Milligan et al 1975, Ingelby and Gershon-Cohen 1960), direct measurements of body water using an isotopic method have shown no differences between controls and mastalgia patients on the 5th and 25th days of the cycle (Preece et al 1975). Double blind

trials of diuretics in patients with premenstrual tension syndrome (with breast pain) have generally shown no benefit over placebo (Reeves et al 1971, Jordheim 1972, Mattson and Schultz 1974). However one double blind study did show significant improvements in mood and breast swelling on metolazone in patients with PMT who were selected for therapy on the basis of proven water retention of at least 3 pounds per cycle (Werch and Kane 1976). Thus diuretics have no rational basis in the treatment of breast pain, and have been shown to be ineffective in controlled studies of breast pain associated with PMT, although no controlled studies in patients selected purely on the basis of mastalgia have been reported.

Other agents such as vitamins have been suggested to be of value in therapy, and vitamin B1 has been recommended for mastalgia (Cuningham and Wyse 1959, Renwick 1979), but no controlled studies have been published. Vitamin B1 is purported to work by detoxification of androgens in the liver. Vitamin B6 (pyridoxine) has also been used in treating breast pain when it has been part of the premenstrual syndrome but once again there is no good evidence that it works (Stokes and Mendel 1972), indeed a recent controlled trial showed it was no better than placebo (Smallwood et al 1986). Studies of serum prolactin levels while taking pyridoxine have shown no change on the vitamin taken orally for 3 months (Goodenow and Malarkey 1979, Peters et al 1978). Further, pyridoxine failed to suppress lactation or reduce prolactin levels in normal post partum women (Canales et al 1976). Vitamin E was reported to give excellent clinical results

in a study published in 1965 (Abrams 1965) and this therapy was enthusiastically taken up by an endocrinology group in the U.S.A. who reported favourable preliminary results of a double blind trial, and correlated response with a decrease in serum dehydroepiandrosterone levels (Solomon et al 1972, London et al 1981, Sundaram et al 1981). However,completion and analysis of the full double blind trial involving a total of 128 patients showed no differences between placebo and vitamin E, again emphasizing the critical necessity for controls in studies of mastalgia therapy (London et al 1985). This study showed no effect of Vitamin E even at 3 different dose levels (150, 300 and 600 IU) and there were no significant changes in serum oestradiol, progesterone, testosterone or dehydroepiandrosterone sulphate levels.

The role of thyroid hormones in breast disease is a controversial issue and a recent review suggests no clearly defined relationship (Vorherr 1978). Kalache also reported a large case control study and found no clear relationship between thyroid disease and breast cancer (Kalache et al 1982). Administration of thyroid hormone has been claimed to be of value in 2 papers describing the treatment of breast pain (Daro et al 1964, Estes 1981), but both studies were conducted without controls and the Estes study allowed concomitant treatment with vitamin E and dietary xanthine restriction, both of which could give substantial placebo effects. The paper by Daro et al mentions a study of 286 patients with hypothyroidism and cystic mastitis who were treated with thyroid hormone, but no data is

given although the patients were said to be improved (Daro et al 1964). Peters reported the treatment of 18 cyclical mastalgia patients with thyroid hormones and found normalisation of an exaggerated prolactin response to TRH and improvement in breast pain in 16 of the 18 patients (Peters et al 1985). Although a pre-treatment placebo period was used, the trial was not double blind and 13 of the 18 patients had goitres, compared with 1 of 3 in the control group, even though their thyroid function was said to be normal.

Since the epidemiology of breast cancer has shown a clear relationship to obesity, it was natural that the role of diet in benign breast disease would be of considerable interest. The idea of dietary restriction of methylxanthines such as coffee,chocolate and cola,was suggested by a group led by Professor J. Minton in Columbus, Ohio and this group has published work to support both the biochemical basis and the therapeutic efficacy of this approach (Minton et al 1979, Minton et al 1981). The mechanism of action of this approach is suggested to be increased cAMP activity at the level of the breast cell, induced by an increase in circulating catecholamines (Minton et al 1981). These series of studies although elegantly demonstrating biochemical differences, suffer from the common problem of lack of controls in the assessment of therapy and the difficulty of ensuring compliance with the methylxanthine restriction regimens, which is inherent in any dietary restriction study. Also the postulated mechanisms by which methylxanthines may affect the breast cell are hypothetical. A

further difficulty is that recent collaborative work from Cardiff and Minton's unit has shown that British women with cyclical mastalgia have identical serum methylxanthine levels compared with asymptomatic age matched control women (Harrison et al 1987).

Epidemiological evidence also contradicts this simple view of excess methylxanthine intake in patients with benign breast disease. Several case control studies of the diet of patients with benign breast disease have concluded that there is little or no association between methylxanthine intake and breast disease (Lawson et al 1981, Lubin et al 1985, Heyden and Fodor 1986). The study by Lubin et al matched 854 cases of histologically diagnosed BBD with 754 surgical controls and 723 neighbourhood population controls and found no relationship with coffee consumption and BBD. These studies and the direct measurement of serum methylxanthines reported by Harrison et al (Harrison et al 1987) suggest that coffee drinking is not a major aetiological factor in mastalgia. A recent paper does give some support to Minton's hypothesis as it demonstrated an increased risk of histologically proven atypia in heavy coffee drinkers, but, strangely, the other histological subgroups of benign breast disease were not increased (Boyle et al 1984).

Dietary factors have been thought to play important roles in many diseases and the increasing understanding of the synthesis of substances that mediate the effects of hormones at the cellular level, has lead to research on the prostaglandins

and their derivatives. The actions of prolactin are thought to be modulated via the prostaglandins. Prostaglandins were discovered by noting that semen caused contraction of uterine muscle and the substance was given the name prostaglandin in 1936 (Von Euler 1936). About the same time it was discovered that some fats were essential for proper growth and nutrition, and the 18-carbon fatty acid linoleic acid was identified as one of the essential fatty acids (Burr and Burr 1930). It was then rapidly found that in man the metabolic pathway of linoleic acid involved conversion to dihomo-gamma-linolenic acid and thence to arachidonic acid and it is now known that this latter substance is the starting point for the synthesis of prostaglandins (Samuelson et al 1971). The bewildering variety of actions of these remarkable compounds has led to their experimental use in many conditions. Rolland et al found that women with "benign mastopathy" showed large rises of prostaglandin E2 which correlated well with thermographic changes during the menstrual cycle (Rolland et al 1981). Recent work on the physiological actions of prolactin led to the suggestion that alteration of the intake of essential fatty acids, especially linoleic acid, might cause altered expression of the action of prolactin in the breast (Horrobin 1979). Horrobin also found that schizophrenic patients undergoing dietary supplementation with linoleic acid, noted reduced breast nodularity (Horrobin - personal communication 1979). This chance clinical observation led to the exploration of this hypothesis by the setting up of a controlled trial of dietary supplement with evening primrose oil as later described in this thesis.

Many other physical or mechanical treatments have been advocated for relief of breast pain, ranging from the simple supporting brassiere (Geschickter 1945, Wilson and Sellwood 1976) to the aggressive alternative of subcutaneous mastectomy (Blevins 1981, Pennisi and Capozzi 1973). Assessment of these therapies is extremely difficult because no placebo control group is possible and drug trials have typically demonstrated placebo responses of up to 40%. The more aggressive surgical treatments do however demonstrate that some physicians consider that some women suffer pain severe enough to embark on these major treatments. Radiation was briefly in vogue for the treatment of the symptoms of chronic mastitis (Keynes 1923), but the carcinogenic risks soon consigned this therapy to the history books.

This historical review clearly shows that, despite a great deal of effort and study over many centuries, the treatment of breast pain and nodularity had remained unsatisfactory. The major difficulty with previous studies of therapy for mastalgia was that no controls were employed and the methods of assessment of response were not detailed. These 2 requirements are a sine qua non in any trial of therapy, because the symptoms are subjective whatever "scientific" methods of assessment are employed, and thus the placebo effect has to be measured. The assessment of several new and unique agents using properly controlled trials forms the basis of this thesis.



Figure 1.1 Sir Hedley Atkins
from 'Memoirs of a Surgeon',
Springwood Books 1977
(with permission)

CHAPTER TWO

TRIAL DESIGN AND PATIENT SELECTION

2.1 INTRODUCTION

2.2 PATIENT SELECTION

2.3 TRIAL STRUCTURES AND PLACEBOS

2.4 ASSESSMENT OF RESPONSE TO TREATMENT

2.5 TRIAL DROPOUTS

2.1 INTRODUCTION

As already noted in the historical review, there are 2 principal problems which must be solved when setting up a study of drug therapy on a wholly subjective symptom such as pain. The first is the control of the placebo effect and the second is the method of recording the changes in the symptoms.

The concept of the placebo, (derived from placere - "to please") is not new and was the predominant mechanism of treatment in the days of Hippocrates and Galen, since most medicines contained large numbers of ineffective, if not dangerous, ingredients. Galen's own pharmacopoeia contained 820 substances, all worthless in terms of modern pharmacology (Shapiro and Morris 1978). Treatments such as cupping and leeches were distasteful or painful and have no basis in physiology, yet patients continued to come to the physician and many were apparently helped. We now know that the apparent effectiveness of these procedures and medications was largely due to the placebo effect. Before modern pharmacology it was impossible to distinguish between a placebo and a non-placebo. The original definition of the word placebo was "a common place method or medicine", as defined in the 1795 edition of Motherby's New Medical Dictionary (Motherby 1795), thus recognising that most medicines of that time worked simply by the placebo effect. The 1982 Supplement to the Oxford English Dictionary now defines a placebo as :

"a substance or procedure which a patient accepts as a

medicine or therapy, but which actually has no specific therapeutic activity for his condition or is prescribed in the belief that it has no such activity ". (Oxford English Dictionary 1928).

This definition implies that a placebo is given deliberately for a therapeutic effect but in the context of a controlled clinical trial a placebo may be defined as :

"a substance when used as a control in experimental studies, is without specific activity for the condition being evaluated " (Shapiro and Morris 1978).

This does not imply that placebos always have a positive effect on the symptom being treated; they may equally have a negative or no effect. Also the definition does not mean that specific agents do not also have placebo effects - the overall effects of a specific therapy represent the sum of placebo and non-placebo effects. It is often thought that placebo effects occur only in conditions with a predominant psychological overlay, but placebo effects have been documented in cancer (Klopfer 1957), in surgery (Diamond et al 1960) and optometry (Carter and Allen 1973). Placebos have also been shown to be addictive (Vinar 1960) and to be capable of reversing the action of potent drugs (Hagens et al 1957, Wolf 1959). These studies show the diverse and powerful effects of placebos and reinforce the concept of placebo control in studies of subjective conditions.

Placebo responses are not as simple as they appear at first sight as many different variables effect the response of an

individual to a placebo. Some patients react to all placebos given to them and are known as "placebo reactors", whereas some only react to certain placebos. This idea is supported by a fairly consistent placebo response in many different studies, put at 35% by Beecher in a review of 15 studies involving 1,082 patients (Beecher 1955). This concept has been challenged by others, and studies are reported showing placebo response rates varying from 0 to 100% (Hass et al 1963, Parkhouse 1963, Shapiro 1964). The current view is that there is no consistent "placebo reactor" and attempts to correlate placebo response with demographic variables such as age and sex, or personality variables such as suggestibility and intraversion/extraversion, have failed to show any consistent relationship (Shapiro and Morris 1978). These are further cogent reasons for using a control group of identically selected patients who are randomly given an identical placebo in each study. Also, any additional treatment the control arm receives should be identical to that of the active treatment group, as situational variables such as staff attitude and treatment setting have been shown to influence the placebo response. It has been shown that negative staff attitudes to the concept of a placebo controlled trial have reduced the predicted effects of active medication (Sabshin and Ramot 1956, Volgyesi 1954) and conversely, positive staff attitudes such as displaying interest enhance the placebo effect. This particular point is of crucial importance in the setting of a Mastalgia Clinic, as the patients are seen by secondary referral from the main breast clinic, and have had the possibility of cancer ruled out (a powerful placebo). They are

then reviewed in a clinic with time set aside to discuss their symptoms in detail, and their pain is assessed by pain charts and other documentation. This extra attention is an obvious placebo, and thus, in testing the value of a new drug, careful controls are required, and were used for these reasons in the studies described in this thesis.

The foregoing considerations referred to the patients attitude to treatment, but of equal, if not greater, importance is the clinician's attitude and expectations towards the patient and the treatment. It is obvious that an investigator has a vested interest in the success of his experiments and many conscious and unconscious biases occur in so-called "objective" work. It has been shown that the interest of the investigator effects the results of surgery in dogs, (Wolf 1962), gastric acid secretion (Engel et al 1956), metabolic changes (Schottstaedt et al 1959) and galvanic skin responses (Dittes 1957). In a recent study physicians were asked to rate patients in terms of likeability, attractiveness and their suitability for treatment, and it was shown that each of these 3 variables strongly correlated with the physician's rating of the patient's improvement on treatment (Shapiro et al 1976). The negative side of this response, where the physician does not like the patient or feels that she is not suitable for treatment, has also been demonstrated and may explain the poor results of mastalgia treatment over the years, and has perhaps led to the label "neuroticism" so commonly employed by many clinicians. Despite all of the varying responses of placebo therapy, three recent

editorials have wholeheartedly supported the concept of placebo-controlled trials, especially where no standard therapy exists (Wilhelmsen 1979, Lasagna 1979, Feinstein 1980).

However, placebos will only control one element of the controlled trial, but will not prevent the bias of the physician's attitude to the patient on active therapy compared with the patient on placebo. This bias can be expressed either in unconscious signals which the investigator conveys to the patient, or in the interpretation of the data resulting from the study. This effect is clearly present, as it has been shown that uncontrolled studies report success much more often than controlled studies (Foulds 1958, Fox 1961). This was shown very nicely in the studies of vitamin B6 and E, where uncontrolled studies showed very good response rates, but double blind studies carried out by the same investigators using similar doses showed disappointing results compared with placebo therapy (Stokes and Mendel 1972, London et al 1985). Thus, a further control, that of double blinding, should be introduced in studies where the data is subjective i.e "soft data". If death or a serum measurement is the sole assessment of a therapy, then there is little chance that the data will not accurately represent the true situation, except for errors in transcription or fraud, but if change in a symptom is the end point, then considerable observer bias could be introduced. For these reasons the introduction of double blinding will control both the patient's and the investigator's preconceptions regarding therapy whether it be placebo or active. Then, provided the method of symptom assessment is identical for

both groups, it should matter little if the data is "soft", although, as in the present studies, several different recording methods can be used to provide some cross correlations.

One further control is required, that of selection of patients. It is clear that prior knowledge of who will receive the active or the placebo treatment could influence the treatment given, usually, again, as an unconscious bias. Thus it is clear that as homogeneous a group as possible should be selected for study, and cases of misdiagnosis or lacking the syndrome or symptoms being investigated, should be excluded. The method of allocating treatment should then depend on chance, which will prevent any selection bias in therapy. Randomisation also ensures that any other confounding variables would be distributed evenly throughout the groups under study. The homogeneity of each patient group is especially important when crossover trial designs are being used. (Pocock 1983).

2.2 PATIENT SELECTION

The patients included in these trials were referred by their general practitioner to the University Department of Surgery Breast Clinic in Cardiff for investigation and diagnosis of their breast disease. This clinic sees around 1,500 new cases per annum of whom about 1 in 12 are suffering from breast cancer. After history taking and clinical examination, patients older than 25 years underwent mammography and a clinical diagnosis was then made. Patients with palpable dominant lumps underwent aspiration cytology and/or Tru Cut needle biopsy. Patients with a diagnosis of benign breast disease were then discharged from the Breast Clinic, unless they were complaining of severe mastalgia which continued despite adequate reassurance. There are several distinct causes of breast pain and they can be broadly divided into physiological , secondary and idiopathic as shown in Table 2.1. The physiological causes and secondary causes were generally discovered in the initial assessment made in the Breast Clinic, and appropriate action taken. Several patients with referred pain from the chest wall or spine were seen in the Mastalgia Clinic. These patients were generally suffering from cervical spondylosis or the painful costo-chondral junction syndrome (Tietze's syndrome - Tietze 1921). Other clinics studying mastalgia have reported a high incidence of referred pain from the cervical spine (Nottingham Breast Clinic), but only a small number of the Cardiff patients fell into this category (Preece et al 1976), since these patients were generally identified in the Breast Clinic. Patients with severe persistent mastalgia were referred

Table 2.1 - Causes of Mastalgia

(a)	Physiological	- Puberty, gynaecomastia, pregnancy
(b)	Secondary	- Infection (abscess)
		- Trauma (fat necrosis/haematoma)
		- Cancer
		- Referred pain (i) intrathoracic
		(ii) chest wall(Tietze)
		(iii) spine
		(iv) abdomen
		- Drugs (exogenous oestrogens or androgens)
(c)	Idiopathic	- Cyclical Mastalgia
		- Non-cyclical Mastalgia

to the Mastalgia Clinic for further investigation. The clinic is run by a full time surgical research fellow and held for a half-day each week with the help of a nurse volunteer and an appointments system. The patients were prospectively documented on a mastalgia proforma detailing the clinical features of the pain and the findings on clinical examination and mammography. More recently, assessment of the severity and duration of the pain has been made on specially designed pain charts (Fig 2.1) which are printed on card, with a record of 6 months. An initial record is kept for 2 months and the patients are then reviewed to establish the total days of pain per cycle and the relationship to the menstrual cycle. This enables the patients to be divided into cyclical and non-cyclical pain patterns to allow stratification of these 2 patterns in therapeutic trials. Previous review of 232 patients in the Mastalgia Clinic had allowed these 2 patterns to be described (Preece et al 1976). Briefly the clinical features are:

(a) Cyclical pattern (Fig 2.2)

Premenopausal -mean age 33 yrs

Pain predominantly in the luteal phase of the cycle

Pain usually relieved by menstruation

Nodularity commonly present

Radiation down arm commonly present

Symptoms - "Heaviness and tenderness to touch"

Daily Breast Pain Chart

Name

Record the amount of breast pain you experience each day by shading in each box as illustrated.

■ Severe pain

◪ Mild pain

◉ No pain

For example:- If you get severe breast pain on the fifth of the month then shade in completely the square under 5. Please note the day your period starts each month with the letter 'P'

Please bring this card with you on each visit.

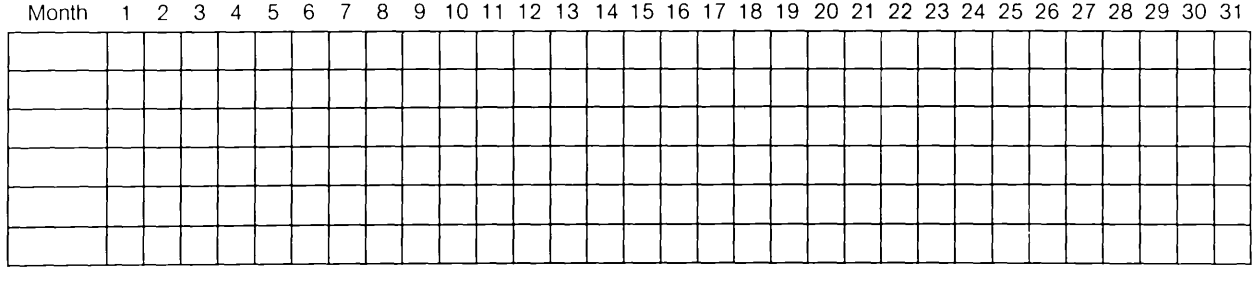


Figure 2.1 The Cardiff Breast Pain Chart

(b) Non-cyclical pattern (Fig 2.3)

Pre and post-menopausal - mean age 43 yrs

Localised pain ("Trigger spot")

No relationship to menstrual cycle

Symptoms - "Burning or pricking pain"

Patients who were aged 21 years or more and had cyclical or non-cyclical pain for at least 7 days per month, and who found the pain interfered with their work, social life or marital relationships were considered suitable candidates for therapy. The duration of pain was determined from the visual linear analogue (VLA) and pain charts which had been completed for at least 2 months prior to trial entry. The presence of severe pain was therefore the sole criterion for trial entry, and the presence or absence of nodularity did not influence this decision but was noted on the trial proforma. No patient had a dominant lump as these patients had been excluded at the initial Breast Clinic visit and biopsy performed to obtain a diagnosis.

Full informed consent was obtained from each patient before entry into any of the trials, and the spirit of the Helsinki declaration (Declaration of the World Medical Assembly, Helsinki 1975 and amended by the 35th World Medical Assembly, Venice 1983) was followed, allowing patients to drop out at any point in the trial if they felt unhappy. Patients were told that they would receive treatments in a random order and that they could receive an inert tablet as part of the treatment. They were

not warned of crossovers but possible side effects were mentioned, and the patients were given a purpose-designed form for reporting side effects and were asked not to mention side effects to the clinician unless they were severe enough to cause trial dropout. The patients were examined in the Mastalgia Clinic at least once each month by the same research fellow, who checked tablet compliance and distributed the symptom assessment forms. They were seen in late luteal phase, between the 20th and 25th days, when symptoms were expected to be maximal. The patients also continuously completed the pain chart in the danazol and EPO trials throughout the period of therapy.

The trials were run sequentially with the bromocriptine trial running from 1977-79, the danazol trial from 1979-1981, and the evening primrose oil trial from 1981-84. Patients were entered after the severity of their pain had been ascertained and informed consent had been obtained. Blood sampling for hormone estimations was performed pre-trial and at each clinic visit. The trial documentation was stored until the closure of the trial, when the scoring and statistical analyses were carried out.

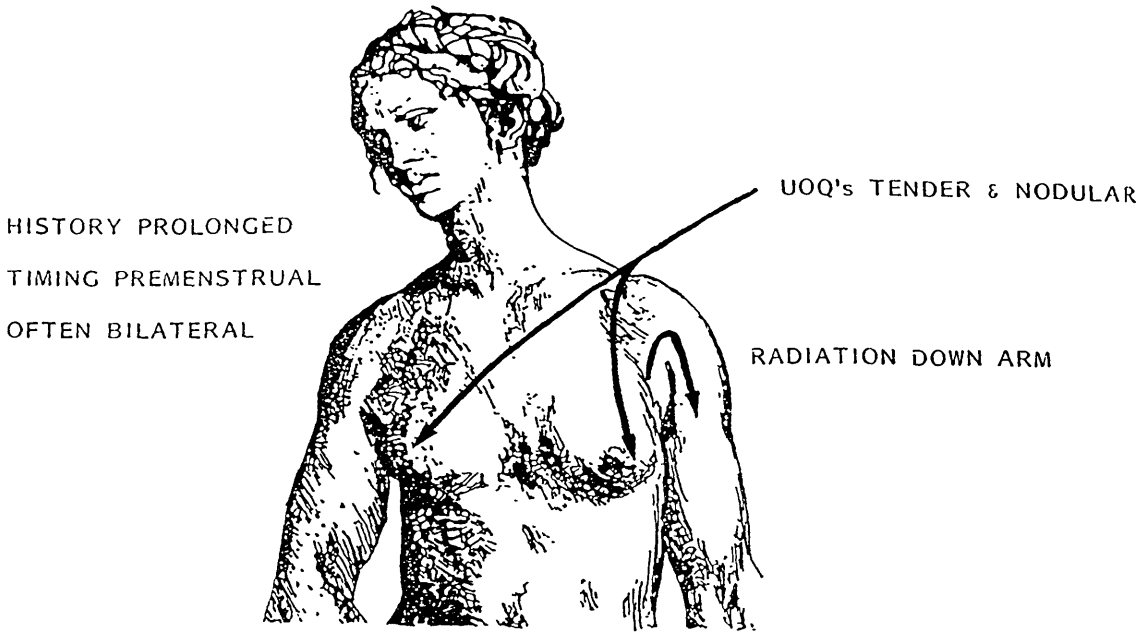


Figure 2.2 FEATURES OF THE CYCLICAL PATTERN OF MASTALGIA

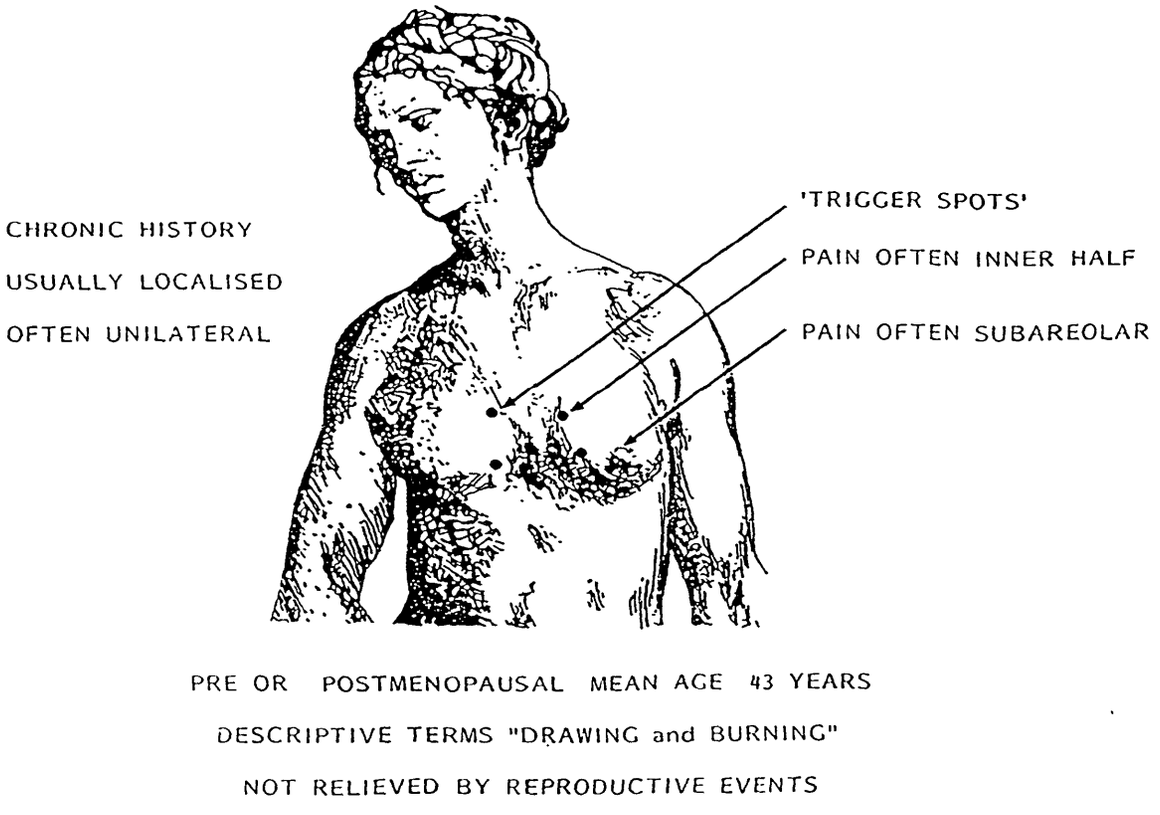


Figure 2.3 FEATURES OF THE NON-CYCLICAL PATTERN OF MASTALGIA

2.3 TRIAL STRUCTURES AND PLACEBOS

As previously discussed in the historical review, the clear need in these studies was to control for the placebo effect and remove selection bias by a randomisation scheme. All 3 trials were pre-randomised in blocks by packing the active drug or the placebo treatment in the pharmacy so that the clinician was unaware of the treatment order. Treatments were packed to ensure an equal distribution of placebo and active treatments throughout the trial (block randomisation). The containers for both placebo and active tablets were identical and the placebos were identical in appearance to the active tablets or capsules. The code for the treatment order was held by the pharmacy in a purpose designed tamper proof 'advent calendar' type chart which allowed individual code break in the event of emergency, but preserved the blindness of the other trial patients. This was accomplished by opening an individual "window" in the card for each patient if an emergency toxic reaction required a code break. The placebos were supplied in each case by the manufacturer of the original active agent (Sandoz Ltd for bromocriptine, Sterling Winthrop Ltd for danazol and Efamol Ltd for evening primrose oil). Lactose was used as a placebo for bromocriptine and danazol, and paraffin oil for evening primrose oil.

The randomization and placebo methods were similar for all 3 trials, but the actual trial structures and assessments were slightly different and are detailed below. The double limbed crossover design was used in the bromocriptine and danazol

studies as it has several advantages over conventional parallel group randomised designs (Pocock 1983, Fleiss 1986). Crossover designs require smaller numbers of patients and give more precise evaluation of the drug under study as each patient acts as their own control. Certain assumptions have to be made in crossover trials. The condition under study should remain relatively stable over long periods, and the treatments used should be short in comparison to the likely duration of the disease (Pocock 1983). In addition the first treatment should not have a prolonged carry over effect as this will make the interpretation of the effects of the second treatment very difficult. For this reason the order of the two treatments is allocated randomly so that half of the patients start on the first treatment and half start on the second treatment. In the bromocriptine and danazol trials the chronicity of mastalgia has been assumed, although it is known that a small number of patients will achieve spontaneous remission, but these should be uniformly distributed by the randomisation process. Two other interactions need to be considered: those of the period effect which occurs when the same treatment gives different results depending whether it is the first or second in treatment order, and treatment interaction effects which are exemplified by the carry over effect (Fleiss 1986). Both of these effects can be checked by comparing the mean difference and the means for the two treatment orders and performing a t test in the case of parametric statistics, but for non-normally distributed responses such as linear analogue data a Wilcoxon test is appropriate as described by Koch (Koch 1972) or Hills and Armitage (Hills and Armitage 1979).

Although crossover designs are useful in chronic studies and minimise the number of patients needed, the resulting data needs to be analysed with care.

(a) Bromocriptine Trial.

This was a double blind, randomized trial with one group of patients randomized to receive bromocriptine or placebo (Fig 2.4). These treatments were continued for 2 menstrual cycles at which point, unknown to the patient, a crossover occurred to the opposite therapy, which was continued for a further 4 cycles. Thus, one group received 2 cycles of bromocriptine therapy followed by 4 cycles of placebo therapy while the other group received 2 cycles of placebo therapy followed by 4 cycles of bromocriptine therapy. Menstrual cycles were chosen as the treatment unit, as the recording cards ran from one menstrual period to another, and it was known that bromocriptine would not effect the length of the menstrual cycle. The crossover point was chosen after 2 cycles so that 2 different active treatment periods (2 months v. 4 months) could be compared to see if there was any benefit of a longer duration of treatment. In addition, in this study the patients were stratified before randomisation into cyclical and non-cyclical pain. The dose of bromocriptine was 2.5 mgs BD given in an incremental scheme (as detailed in Chapter 3) in order to reduce side effects on commencing therapy. The placebo was given in an identical incremental scheme to maintain the blindness.

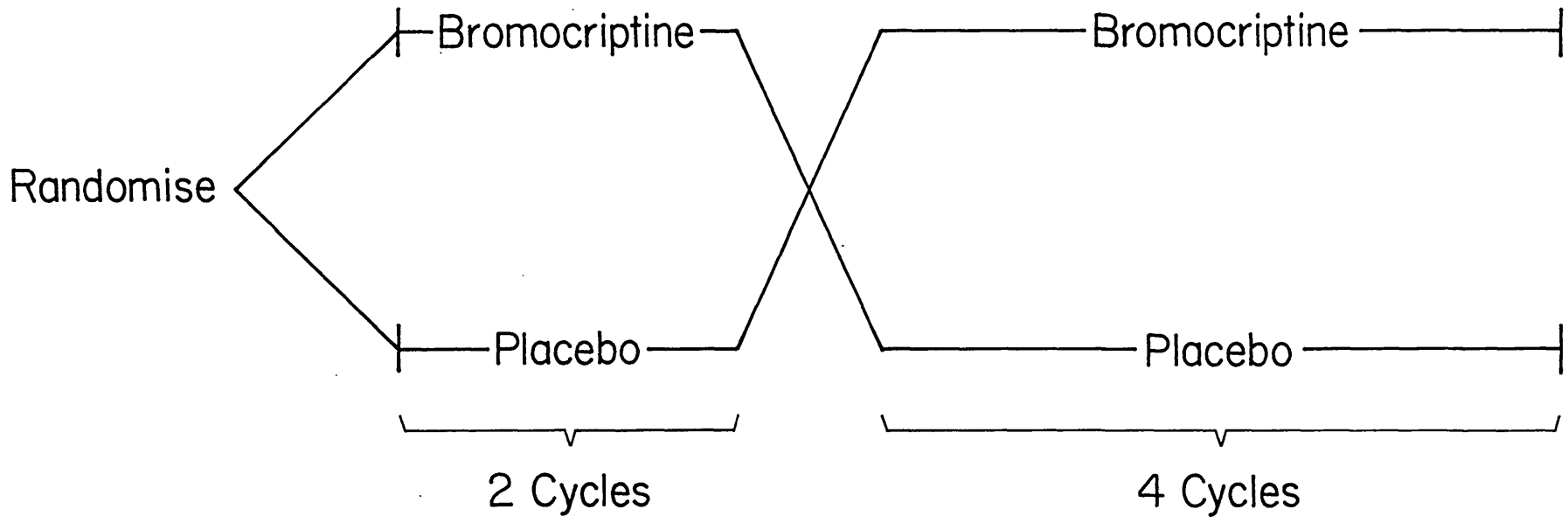


Figure 2.4 THE STRUCTURE OF THE BROMOCRIPTINE TRIAL

(b) Danazol trial.

This was also a randomized, double blind crossover structure but with a crossover at the mid point (Fig 2.5). The total trial duration was 6 months with an initial 3 months therapy randomized between danazol and placebo and a crossover point after 3 months. Thus one group received danazol for 3 months followed by 3 months placebo, and the second group received 3 months placebo followed by 3 months danazol therapy. This structure was chosen as previous uncontrolled trials of danazol had suggested that more than 2 months therapy was needed to see the maximal therapeutic effect and thus the 2 active treatment periods needed to be at least 3 months each. The first 6 patients received a dose of 400 mgs daily but this was reduced to 200 mgs for the remainder of the patients, as it had emerged from newly published pilot studies that a 200 mgs dose was sufficient for cases of benign breast disease. This change in dosage was made without discovery of the random code by the investigator, as it was done by the pharmacy staff. Patients were treated on 30 day "months" and not on menstrual cycles as it was likely that the active therapy (danazol) would effect the menstrual cycle. Only patients with cyclical pain were entered into this trial as the previous bromocriptine trial had shown no response in the non-cyclical patients.

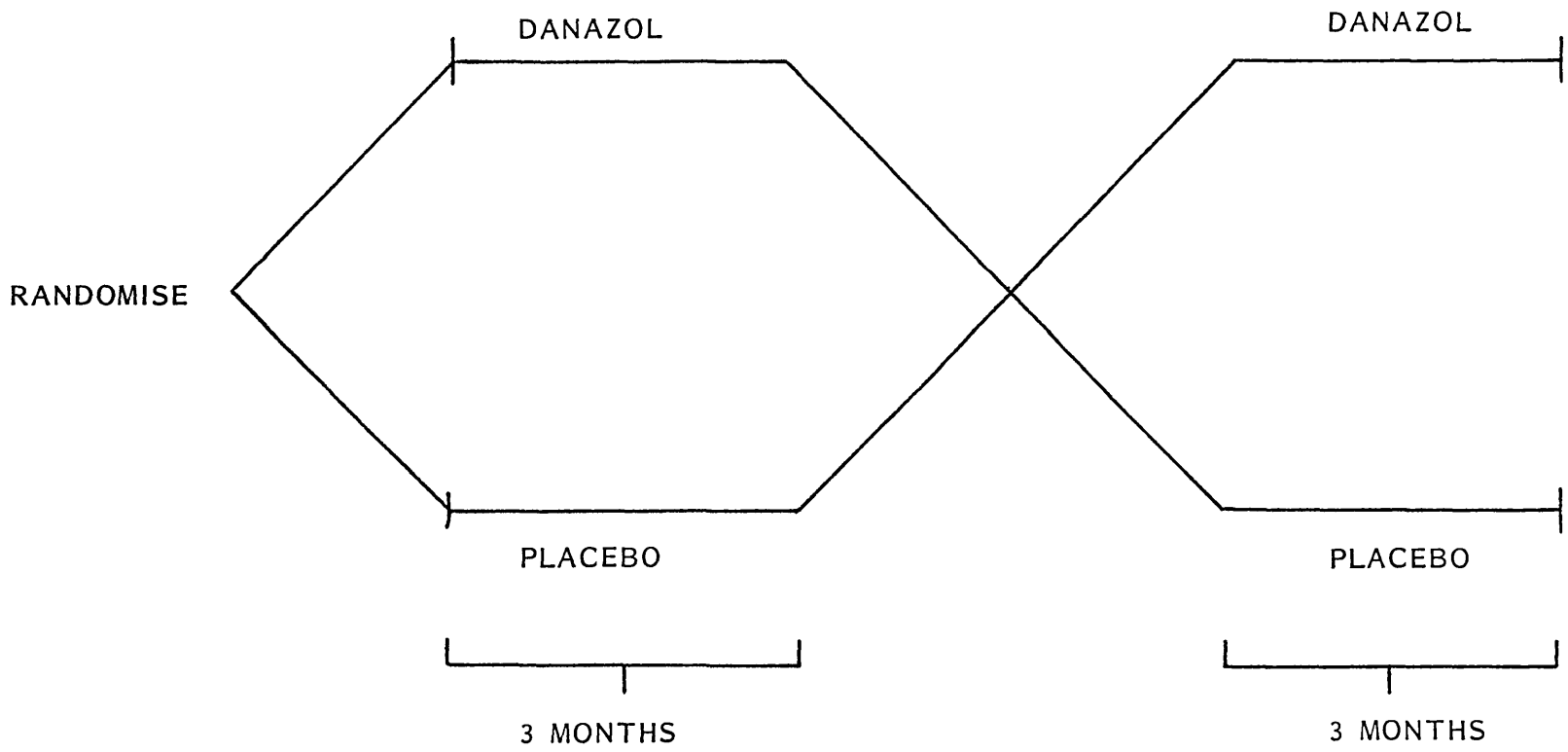


Figure 2.5 STRUCTURE OF THE DANAZOL TRIAL

(c) The Evening primrose oil trial.(EPO)

This also was a randomized, double-blind study with 4 months duration of therapy. The trial structure differed from the preceeding trial in that those patients who were randomized to start on evening primrose oil did not crossover but remained on evening primrose oil for the duration of the study (Fig 2.6). The patients who were randomized to start on placebo crossed over to evening primrose oil after 2 months. The trial structure was therefore a double limbed structure with a single crossover, and was unknown to the patient. The reason for a lack of crossover in the group initially treated by evening primrose oil was that the agent is believed to work by altering fatty acid levels, which is a gradual process and thus a prolonged carry over effect could be anticipated after 2 months therapy with EPO which would make interpretation of the resulting data difficult. Indeed, Fleiss has pointed out that a crossover should not be used when the magnitude and duration of a potential carryover effect is totally unknown (Fleiss 1986). However, the trial structure did give the opportunity of again comparing a 2 months course of active treatment with a 4 months course.

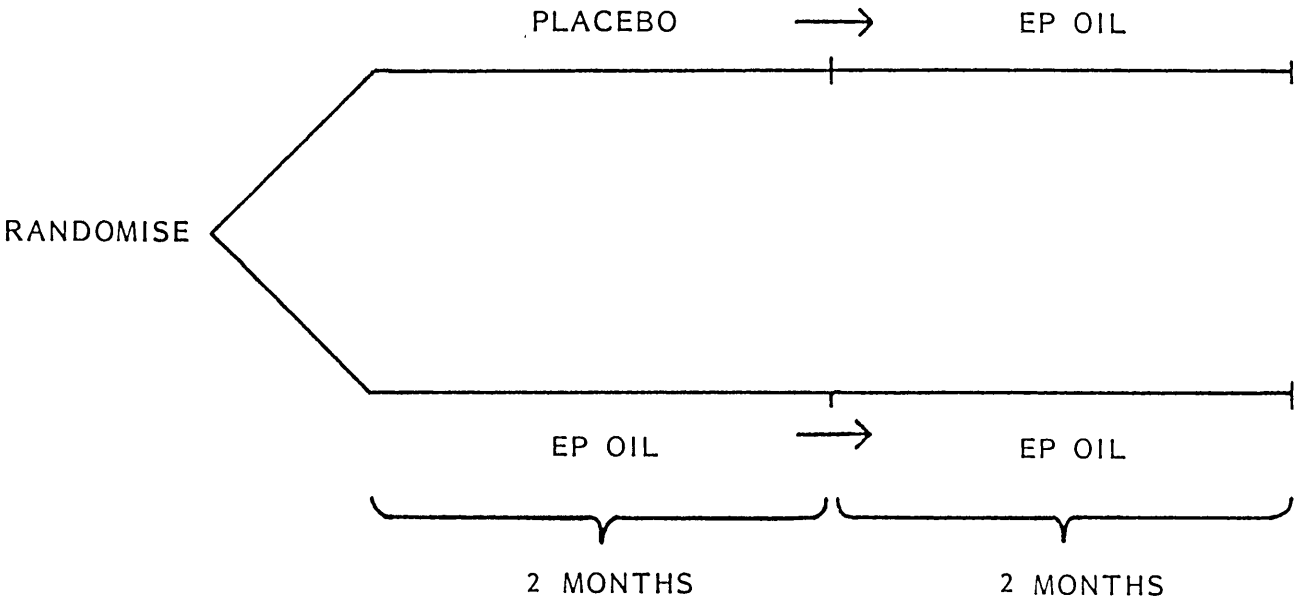


Figure 2.6 STRUCTURE OF EP OIL TRIAL

2.4 ASSESSMENT OF RESPONSE TO TREATMENT

There are considerable difficulties in attempting to measure a subjective symptom such as pain, let alone a change in that symptom at a later date. Most research has concentrated on pain as it is such an overiding symptom of human illness, which is extremely difficult to measure. The early work on measuring subjective feelings came from the rapidly increasing interest in psychology in the early part of this century. Many scales were devised by investigators trying to measure feelings or moods, and the visual linear analogue scale (VLA) and the graphic rating scale were described as early as 1921 (Hayes and Patterson 1921, Freyd 1923). The linear analogue scale consists of a line of defined length with clearly marked ends which are labelled with the extremes of the range of the feeling or symptom to be measured (Fig 2.7).This allows a description of pain, for example from "no pain" to "severe pain", and the subject can mark the line at any point to indicate the point which she feels will represent her state at the time of marking. The score is obtained by measuring from one end of the line to give a numerical reading e.g 35 in fig 2.7. These scales offer several advantages as they can be described in simple language and so are applicable to all sections of the population, they are easy to score and there is no intermediate description on the line which tends to draw the attention of the subject, and so produce a skewed distribution. The addition of intermediate descriptions to the line, known as graphic rating (Fig 2.8), was also described as being useful (Freyd 1923), but this addition causes a maldistribution of

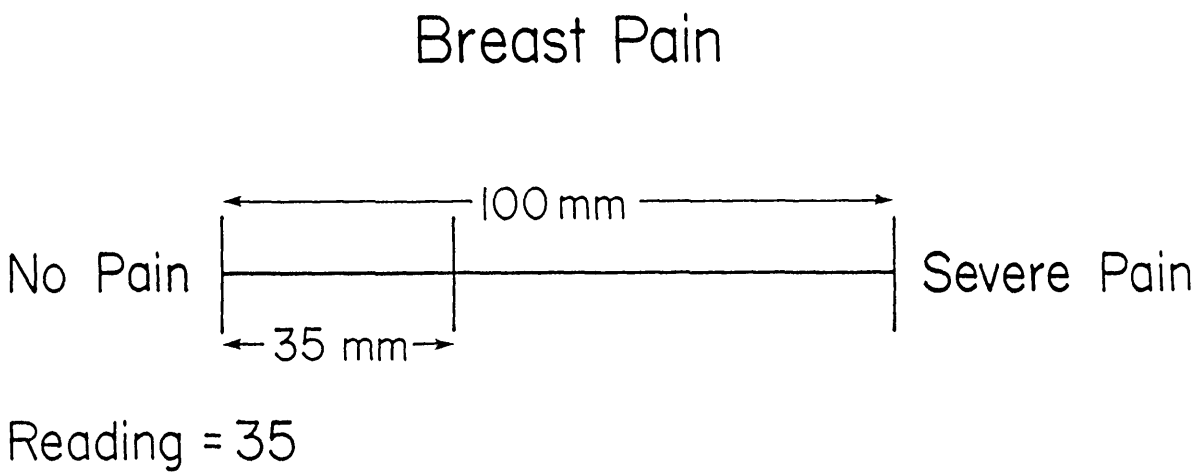


Figure 2.7 A 100 mm length visual linear analogue scale. The patient has placed a mark 35 mm along the scale giving a reading of 35 mm.

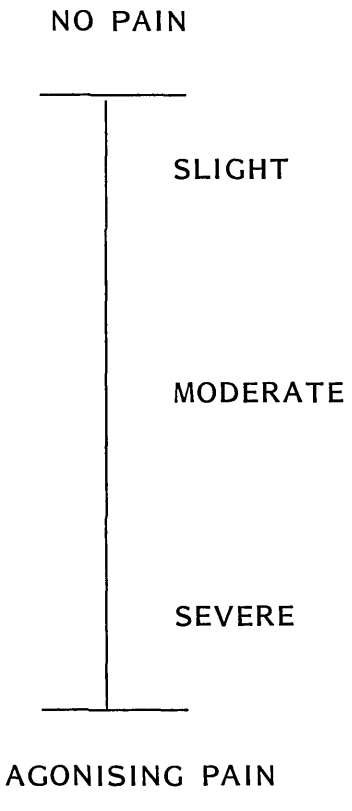


Figure 2.8 THE GRAPHIC RATING SCALE

scores around the intermediate terms (Huskisson 1974). This makes statistical interpretation more difficult for the graphic rating method.

In any case, statistical interpretation of the scores obtained from VLA scales requires care as the scores are not distributed normally as shown by Huskisson (Huskisson 1974), and scores are not directly comparable from one patient to another (Aitken 1969). This is fairly obvious as pain is a personal psychological experience and a mark on the same point of a scale by 2 different individuals does not imply they have the same "amount" of pain. Thus, the numerical scores obtained from the VLA scale should ideally be used to compare the scores of individuals with some other objective measure, or the initial scores of the individual should be compared with his score after some change in treatment or experimental conditions. The ideal comparison is a within-patient analysis, where each patient's VLA score on placebo therapy is compared with his VLA score on active treatment and with the pretrial score; this is the method of analysis that has been employed in the studies detailed in this thesis. The non-normal distribution of the scores can be overcome by using an arcsin transformation which produces an approximately normal distribution, so allowing the use of parametric statistics (Aitken 1969). The alternative is to use non-parametric statistics (Siegel 1956), which do not assume a normal distribution of the data. In the studies described in this thesis, the VLA scores for each patient were compared before and during therapy using Wilcoxon's matched pairs signed ranks test.

This test is appropriate for paired ordinal data such as that derived from VLA scores. A detailed example of the calculations required to perform this test is shown in appendix I. Using the VLA system Aitken showed good correlation between increasing airway resistance in a breathing circuit measured in inches of water pressure, and the subject's subjective feeling of dyspnoea (Aitken 1969). Good correlation has also been shown between patient and observer in a trial of poldine in dyspepsia (Hunt and Wales 1966), in which the doctors marked their estimation of the patient's symptom reduction on VLA scales after interviewing the patient. Revill et al found that a 10-20 cm line gave less variable results for pain measurement than a 5 cm line, and they concluded that a VLA was a suitable method of recording and recalling labour pain (Revill et al 1976). The VLA system has also been used to measure the response of post-operative pain to analgesic drugs (Harmer et al 1983, Rutter et al 1980).

Other systems of recording pain have also been used especially for recording the response to therapy by the clinician. The simple descriptive pain scale is popular, and was originally described by Keele who devised a four point scale grading pain as slight, moderate, severe and agonising (Keele 1948), but since agonising pain is rare in the context of chronic pain, this term has been dropped by most investigators. Often the term "mild" is used instead of slight and the 3 point scale of mild, moderate and severe has become the standard scale (Hewer et al 1949) Fig 2.9. The disadvantage of this scale is its simplicity, as there are so few intermediate points to choose. A

patient with mild pain can only have one degree of improvement, "no pain", which is unlikely in the context of chronic pain. For these reasons most authors have preferred the more sensitive VLA system (Huskisson 1974, Aitken 1969, Zealley and Aitken 1969, Bond and Lader 1974). As a further check in the studies described in this thesis, the clinician completed a simple descriptive pain scale at each clinic visit so that a correlation could be drawn between the VLA score derived from the patient and the pain scale derived from the clinician. Frank et al compared a five point pain scale with a VLA scale, and showed a reasonable correlation between the two scales and also between a picture scale (Frank et al 1982). In these studies, the terms mild, moderate and severe were used as descriptive labels. Again as the data derived from the simple scale are in the form of an ordinal or ranking scale, and a normal distribution cannot be assumed, these data were analysed by non parametric statistical tests.

The third form of assessment was the pain chart (Fig 2.1) which was used for the danazol and evening primrose trials. These charts were designed specifically for these studies and show both the severity and timing of the pain relative to the menstrual cycle. These were completed daily by the patient, and at the end of the trial the total number of days of pain per month for each patient was calculated for the active and placebo periods of treatment. These were analysed by parametric statistics. These charts proved to be useful as they were easily understood by all the patients and were filled in regularly by nearly all the patients.

3 - POINT PAIN SCALE

NO PAIN	0
MILD PAIN	1
MODERATE PAIN	2
SEVERE PAIN	3

KEELE (1948)

Figure 2.9 THE 3 POINT PAIN SCALE

2.5 TRIAL DROPOUTS

Dropouts in a longitudinal trial pose a problem for the statistical handling of results as they have been randomised and should be considered in the results. This is not difficult in some studies, for example, the treatment of breast cancer, as a dropout is regarded as a treatment failure, which is a valid end-point. However, in a study of chronic pain, no such end point is available, and there are 2 options. One is to allocate the last score of the patient to all of the missed assessments, but this is rather artificial and assumes that the patient's score remains constant, an assumption which is very tenuous if the patient drops out before the crossover point, when the 2nd therapy has had no chance of showing its efficacy. The other approach is to use all of the entered patients in the assessment of side effects, but only use the completed patients for the assessment of efficacy and this is the general approach adopted for these studies. As the Wilcoxon's signed ranks matched pairs test actually compares the same patient before and on therapy, theoretically there is no loss if dropouts are not considered as the comparison is within-patient and not between-patient. Of course, a high dropout rate on one of the therapies does indicate problems of toleration which may be of great importance in the decision to use that particular treatment in clinical practice.

A recent leading article notes the problem of trial dropouts after randomisation, but also makes the point that analysis by completed treatment is a valid exercise as long as

the data on intention to treat is also considered (Anon 1987). The same leading article makes the point that studies with human subjects inevitably cannot attain the scientific rigour of laboratory experiments. An analysis of intention to treat is included for the bromocriptine trial in this thesis as the dropout problem in this trial was greater than the danazol or EPO trials.

Thus, these studies have employed a broad assessment of subjective symptoms using pain charts, simple pain scales and VLA scales. Although there are more sophisticated pain questionnaires such as the McGill Pain Questionnaire, these are complicated, time consuming to complete and use many different words with differing nuances of meaning (Melzack 1975). These questionnaires have mainly been used for evaluation of experimental pain which is markedly different from pathological pain. Melzack, who developed the McGill questionnaire admits that the patients find it complicated and he suggests that they are helped to fill it in by a trained assistant (Melzack 1975). This problem of patient understanding is also met with in the VLA forms, but after careful explanation at the initial clinic visits, most patients can fill in a VLA form without help. For these reasons, the VLA assessment has been preferred in these studies as the principal method of assessment of pain and the other symptoms of benign breast disease.

CHAPTER THREE

THE BROMOCRIPTINE TRIAL

3.1 INTRODUCTION

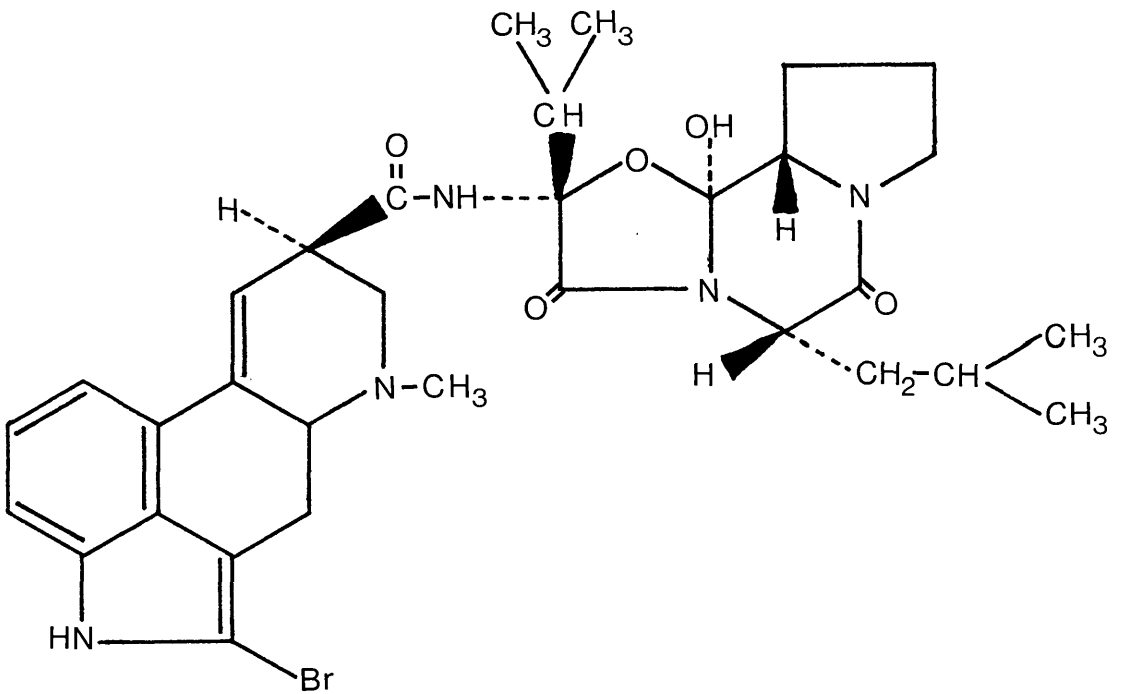
3.2 PATIENTS AND TRIAL METHODS

3.3 RESULTS

3.4 DISCUSSION

3.1 INTRODUCTION

The semi-synthetic ergot alkaloid bromocriptine (2-bromo - 2 -ergocryptine) was discovered to be a prolactin suppressant accidentally, when it was found to inhibit lactation in mice (Fluckiger 1972). It has the general structure of an ergot alkaloid (Fig 3.1) and is known to exert its effect by mimicking the inhibitory effect of dopamine on the pituitary lactotrophs which secrete prolactin, i.e it is a dopamine agonist. The drug exerts this action by stimulating dopaminergic receptors in the brain, thus mimicking the action of dopamine itself. The secretion of pituitary prolactin is under a continuous negative inhibition in humans, and the levels are influenced by many different stimulatory pathways,especially thyrotrophin releasing hormone from the pituitary (TRH), and stress related stimuli. Bromocriptine produces a long acting inhibition of prolactin in all species, including rats (Fluckiger and Wagner 1968), mice (Sinha et al 1974), rabbits (Fluckiger et al 1976), dogs (Meyer and Schutze 1973), cows (Karg et al 1972), goats (Hart 1973), sheep (Niswender 1974) and man (del Pozo et al 1972). This inhibition of prolactin secretion lasts about 12 hours after a single oral dose. The drug is unique in that it is a pure and specific depressant of prolactin, and no effects are apparent on ovulation (del Pozo et al 1973) on the uterus or cardio-vascular system (Sturmer and Fluckiger 1974). Growth hormone is unaffected in goats (Hart 1973), although a minor increase in growth hormone secretion was seen in healthy people (Molinatti et al 1975).



2. Bromo-α ergocryptine mesylate
(Bromocriptine)

Figure 3.1 The chemical structure of bromocriptine

These characteristics allow the specific manipulation of prolactin suppression to be made to the function of the pituitary-ovarian axis, without other endocrine changes, and makes the drug an ideal agent for the treatment of hyperprolactinaemic states.

The evidence of direct involvement of prolactin in the aetiology of benign breast disease is lacking, partly due to the wide variations of the basal levels of this hormone. However the circumstantial evidence for some abnormality of prolactin secretion in benign breast disease is fairly strong, and in teleological terms one would expect prolactin, being the primary lactogenic hormone, to have profound effects on breast function. When direct estimation of serum prolactin by radioimmunoassay had been established, many investigators looked for abnormalities of serum prolactin in breast disease. In general the levels of basal serum prolactin have been found to be within the normal range, or only mildly elevated in cases of benign breast disease (Gorins et al 1984, Malarkey et al 1977, Mussa and Dogliotti 1979, Bischoff et al 1980, Peters et al 1981, Walsh et al 1984a, Wang and Fentiman 1985). The groups of "breast disease" were loosely defined in these studies and may have contributed to some variation, but it is clear in general, that no gross abnormality of basal prolactin is seen in cases of symptomatic benign breast disease. A similar lack of clear abnormality has been demonstrated in breast cancer studies as noted in a recent review (Nagasawa 1979).

Further work has demonstrated that the secretion of prolactin has a large circadian variation with the peak of secretion occuring between 1 and 5 A.M. (Nokin et al 1972, Sassin et al 1972) and this peak is thought to be a sleep related phenomenon (Parker et al 1974). Detailed studies of the 24 hour profiles of prolactin and the night peak has failed to show any abnormality in patients with benign breast disease (Malarkey et al 1977). The patients in the studies performed by Malarkey were well defined, as they had a histological diagnosis of cystic disease or fibroadenomas, but no mention is made of symptoms. One study has shown elevation of basal serum prolactin in carefully defined groups of benign breast disease (Cole et al 1977). This longitudinal study sampled patients at the same time each day for a complete menstrual cycle, and analysis showed that some women with cystic disease, especially if over 30, had a significantly elevated level of serum prolactin. Again, although the differences were statistically different, they were not great in biological terms.

A small number of therapeutic studies of bromocriptine in symptomatic breast disease have been carried out on an empirical basis. They suffer from the common problem of lack of controls, but a small number of open studies did suggest some improvement (Schulz et al 1975, Martin-Comin et al 1976, Palmer and Monteiro 1977). Other evidence pointing to the possible value of bromocriptine therapy came from studies of the premenstrual syndrome,which has breast pain as a major component of its symptomatology. These controlled studies showed overall

improvement in breast symptoms even if the other central nervous system symptoms were unchanged (Benedek-Jaszmann and Hearn-Sturtevant 1976, Andersen et al 1977). In contrast, Ghose and Coppen in a double blind study of the premenstrual tension syndrome, failed to show any advantage of bromocriptine over placebo for breast symptoms (Ghose and Coppen 1977) . In the light of these studies, there was a need for a properly controlled study of the therapeutic role of bromocriptine in the treatment of patients with carefully defined mastalgia.

3.2 PATIENTS and TRIAL METHODS.

(a) Patients

Patients with severe mastalgia, not helped by reassurance, were referred to the Mastalgia Clinic for consideration of therapy. At the initial consultation further details were taken on the standard proforma, and the patients were then further reassured of their benign condition. The technique of completing VLA forms was explained to each patient; they were observed for a further 2 months and asked to complete a VLA form recording their breast and general symptoms in the luteal phase of their cycle between the 20th and 25th days, as determined by completion of basal body temperature charts. The full VLA form is shown in Appendix II and records the following breast-related symptoms: breast pain, breast heaviness, breast tenderness, soreness in the breast, effect of breast pain on sex life and nipple discharge. A further section recorded general symptoms: headache, irritability, depression, bloated feelings in stomach, well being and tiredness. In addition, when the patient was on therapy, a third section recorded the patient's assessment of her response to therapy.

When the patient was reviewed after 2 months, her VLA scores were examined, and she was entered into the randomised trial if she had severe symptoms on the VLA (> 50 mm symptom score) and her mastalgia had lasted for at least 6 months. Patients meeting these criteria invariably complained that the

mastalgia interfered with their lives. Typical problems were pain radiating to the arms making work difficult, inability to hug their children or husband, and avoidance of sexual intercourse because breast fondling caused pain. Patients with a dominant lump requiring biopsy, or who were currently taking any hormonal medication, were excluded from trial entry. There were no other specific contra-indications to bromocriptine therapy. When a patient was deemed suitable for entry, she was given an explanation of the trial and her informed consent obtained. A pretrial blood sample was taken for estimation of prolactin levels, and a routine skull X-ray was performed to exclude a latent pituitary tumour.

Fifty three patients fulfilled these criteria and were entered into the trial. Of these, 38 women completed the full course of 6 months therapy.

(b) Methods

The protocol for this trial was approved by the ethical committee of the Division of Surgery, University Hospital of Wales. After satisfying the criteria for entry and agreeing to take part in the trial,the trial treatment was drawn for each patient. This was done simply by allocating the next trial number which was typed on each of the 6 bottles held in the pharmacy for each patient. The bottles were labelled as shown in Fig 3.2 with only the trial and cycle numbers, so that neither patient nor clinician knew what was in the bottle. The drug

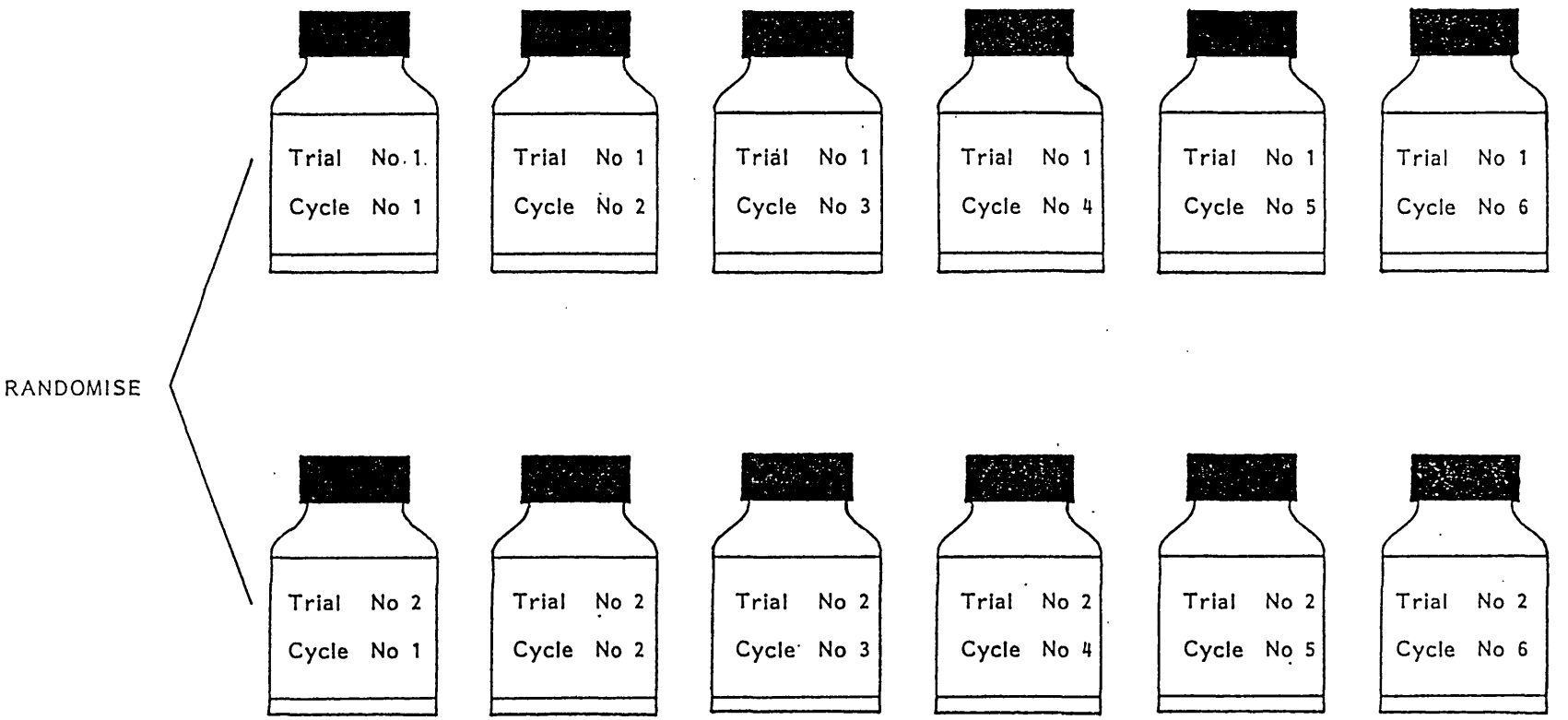


Figure 3.2 The dispensing scheme of the bromocriptine trial showing the 2 randomly allocated limbs.
The labels are the actual labels seen by the patients.

order, whether bromocriptine to placebo or vice versa, was pre-packed according to the randomisation code in the pharmacy. All the tablets were pre-packed before the beginning of the trial,under the supervision of Dr. Brian Evans, Unit Pharmacist, who held the code break in case of emergency.

The dose of bromocriptine was 2.5 mgs twice a day, but this was increased incrementally when bromocriptine was commenced according to the scheme in table 3.1, whereby the full dose was reached at day 16. This dosage regimen was adopted as previous studies had shown a high incidence of side effects when 2.5 mgs BD was taken immediately by patients who were normoprolactinaemic. As bromocriptine was started on the first day of cycle 1 in one group and on the first day of cycle 3 (after the crossover) in the other group, this incremental scheme appeared twice in each patient's regimen (cycle 1 and cycle 3). Written instructions were given out with each bottle to reinforce the explanations given to the patients at the start of the trial. The incremental dose scheme was repeated in an identical manner in the placebo tablet regimens. As the treatment was being given by cycle, sufficient tablets were packed in each bottle to allow for a 35 day cycle. Patients were then seen at least once each month in the luteal phase of the cycle,when the bottle of tablets for the next cycle was issued. At the end of the trial all the bottles were returned and the numbers of tablets remaining in the bottles were counted to give an idea of compliance. Premenopausal patients commenced the first bottle of tablets (cycle 1) on the first day of their period, and continued taking the tablets until

the first day of their next period when they commenced the second bottle of tablets (cycle 2) and so on. The post-menopausal patients in the non-cyclical group took the first tablet on the first day of the calendar month,and continued on to the second bottle on the first day of the next calendar month and so on.

As stated, the patients were seen in the late luteal phase each month between days 25-30 when clinical examination was carried out and two assessments of response to therapy were made. The clinician recorded a score on a simple descriptive scale of breast tenderness and nodularity graded from 1 to 3 as previously described, and also gave a score on a simple scale of treatment response graded : 1=asymptomatic, 2=some response, and 3=no improvement.

Secondly, the patient completed the full VLA detailing breast and general symptoms shown in Appendix II, while out of sight of the clinician, and the form was filed away in the trial packet and was not consulted further until data analysis at the end of the trial. The VLA form allowed the patients to volunteer side effects and no specific oral enquiry of side effects was made in order to preserve the double blindness of the study. Both the clinician's assessment on a simple scale and the patient's assessment on the VLA form were made every month in the luteal phase, until either completion of the 6 cycles of therapy or drop-out from the trial occurred.

Table 3.1 Bromocriptine trial - Incremental Dose Scheme

	AM	PM
DAYS 1-3	-	1.25 mg
DAYS 4-9	-	2.5 mg
DAYS 10-15	1.25 mg	2.5 mg
DAY 16 -> Onwards	2.5 mg	2.5 mg

Statistical Methods

First, the VLA readings of all the patients who completed the 6 months of the trial were analysed by the Wilcoxons matched pairs signed rank test. Then a second analysis was performed on all the randomised patients, to give a measure of efficacy of the drug on an 'intention to treat' basis. Only 2 patients were excluded from this second analysis, as they only had pretrial scores with no other data to make a pair for the Wilcoxon analysis. The patients were analysed in their original randomised groups (placebo -> bromocriptine and bromocriptine -> placebo) and 3 comparisons were made for each symptom using the calculations shown in Appendix I. First, the pretrial value of each individual was compared with the placebo score at the end of placebo therapy in the placebo -> bromocriptine group, and with the score at the end of the bromocriptine therapy in the bromocriptine -> placebo group. Secondly, the score at the end of the placebo period was compared with the score at the end of the bromocriptine period in both the placebo -> bromocriptine and the bromocriptine -> placebo groups. Finally, the mean value for all the placebo scores of each individual were compared with the mean value for all the bromocriptine scores of that individual to see if there were any differences from the analysis using the end of treatment scores.

In addition to the symptomatic assessments each cycle, a venous blood sample was taken from most of the trial patients at each visit (taken between 9 A.M and noon) and stored for later

determination of serum prolactin. The serum was separated immediately and stored frozen at -20 C until assay, which was performed by the Tenovus Institute according to the radioimmunoassay method of Groom (Groom 1977). Comparisons of prolactin means were made by Student's t test. The scores for breast tenderness and nodularity allocated by the clinician were also compared using the non parametric Wilcoxon's matched pairs signed ranks test, using all the data in the treatment periods and comparing these with the pretrial scores.

The progress of each patient was continuously monitored on the purpose-designed form shown in Fig 3.3 (a+b).

BROMOCRIPTINE TRIAL (MASTALGIA)

TRIAL NO:

ADDRESSOGRAPH

Tel No:

GROUP C.P. S.D.

MENOPAUSAL STATUS

PRE POST PERI U/K

DATE OF ENTRY:

DATE OF WITHDRAWAL:

REASON:

HISTORY:

MAMMOGRAM:

PRE-TRIAL INVESTIGATIONS AND DATES

SKULL X-RAY

BLOOD SAMPLES

URINE SAMPLES

VLA FORM

BBT ISSUED

THERMOMETER

Figure 3.3a The bromocriptine trial record form (front)

FOLLOW UP

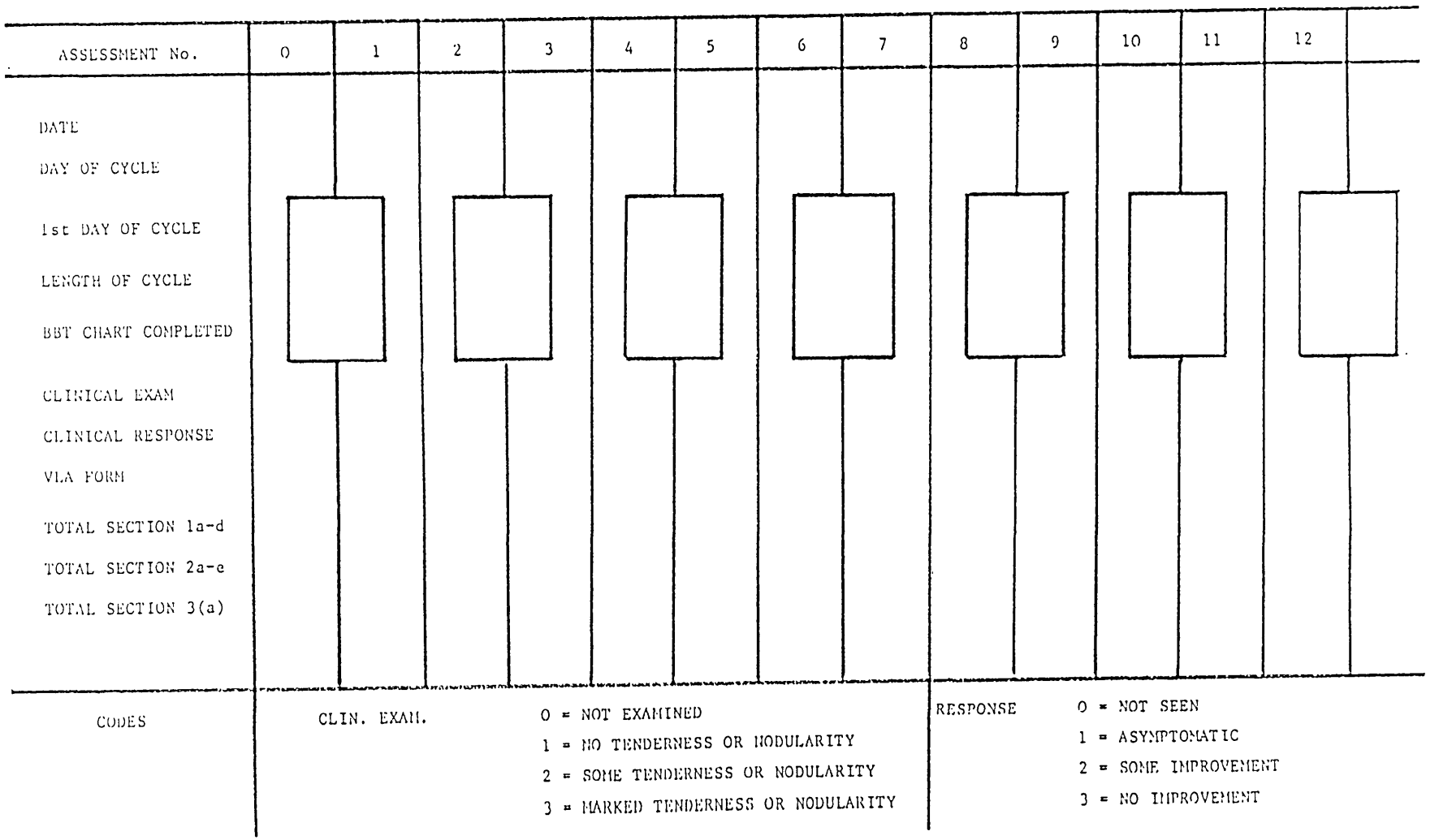


Figure 3.3b The bromocriptine trial record form (back)

3.3 RESULTS.

(a) Patient details.

Fifty three women were entered into the study, each having the criteria for entry defined in section 3.2 of this chapter. Their ages ranged from 19 to 61 yrs with a mean of 35.8 yrs. The clinical features of these patients are given in Table 3.2. Thirty eight women had a cyclical pronounced (CP) pattern of mastalgia and 15 patients had a non-cyclical pattern. As these 2 patterns had been stratified by the protocol, analysis was carried out separately on each group.

The average duration of pain before entry to the trial was a mean of 5 years in the cyclical group, and 3.8 years in the non-cyclical group. The non-cyclical group was older (mean age 42.2 years) than the cyclical group (mean age 32.5 years), as the former group included post menopausal patients as expected from the definition of non-cyclical pain. The length of the menstrual cycle in the menstruating women was similar in both groups, 28.4 days in the cyclical group and 25.7 days in the non-cyclical group. The dropouts and withdrawals during the trial are shown in table 3.3. Nine patients withdrew in the cyclical group: 5 from side effects of therapy, 2 who became pregnant and 2 who suffered intercurrent illness (1 had a cholecystectomy, 1 had acute cholecystitis necessitating hospital admission). This gave an overall withdrawal rate of 24% in the cyclical group, but a true withdrawal rate of 15% (5 of 34) due

Table 3.2 Clinical features of bromocriptine trial patients

	Means ± SD	
	CYCLICAL GROUP	NON CYCLICAL GROUP
	N=38	N=15
AGE (YRS)	32.5±6.2	42.2±9.9
CYCLE LENGTH (DAYS)	28.5±2.8*	25.7±4.6†
PAIN DURATION (YRS)	5.0±5.1	3.8±5.1

* 6 patients had had a previous hysterectomy

† (4 patients had had a previous hysterectomy
(2 patients were post menopausal

Table 3.3 BROMOCRIPTINE TRIAL DROPOUTS

Trial No	Dropped Out	Therapy	Reason
(a) CYCLICAL			
2C	Cycle 1	Bromocriptine	Nausea, headache, anorexia
4C	Cycle 3	Bromocriptine	Intercurrent illness - cholecystitis
5C	Cycle 2	Bromocriptine	Depression, nausea
6C	Cycle 5	Placebo	Became pregnant
9C	Cycle 4	Placebo	Became pregnant
13C	Cycle 1	Bromocriptine	Nausea, spots on face
14C	Cycle 1	Placebo	Faintness (DNA'd)
23C	Cycle 2	Bromocriptine	Intercurrent illness - cholecystectomy
38C	Cycle 1	Bromocriptine	Headaches, nausea
(b) NON CYCLICAL			
2S	Cycle 2	Bromocriptine	Headaches, dizziness (DNA'd)
10S	Cycle 1	Bromocriptine	Dizziness (DNA'd)
11S	Cycle 1	Bromocriptine	Nausea, faintness
12S	Cycle 4	Bromocriptine	Nausea, dizziness
13S	Cycle 2	Bromocriptine	Depression, rash
15S	Cycle 2	Placebo	Hair loss on temple

to drug side effects. In the non-cyclical group 6 patients withdrew, 4 because of side effects, and 2 failed to return to the clinic for review (1 after 5 cycles and 1 on the first cycle). The 2 patients who did not reattend (nos 2S and 10S) were both found to be taking bromocriptine when the code was broken and so must be assumed to have dropped out due to side effects, as both complained of side effects on the visit prior to drop out. This gives an overall dropout rate in the non-cyclical group of 40% which is much higher than in the cyclical group. Even if the 2 pregnant and 2 intercurrent illness patients are considered not to be dropouts due to side effects, this still gives an overall trial dropout due to side effects of 11/49 (22%). The 2 patients who became pregnant both were delivered of healthy babies after uneventful pregnancies.

(b) The patients' assessment of response - VLA forms.

The results of the statistical tests calculated as rank sums are shown for each symptom in tables 3.4 to 3.11. In addition, for illustrative purposes only, the mean scores have been computed for each symptom and are shown in Figs 3.4 to 3.8 for the cyclical patients. The individual VLA scores for each patient are shown in Appendix III. The mean values shown in the graphs in figures 3.4 to 3.8 are purely illustrative as non-parametric tests were used for statistical comparisons of VLA scores as explained in chapter 2. The VLA figures for nipple discharge are not shown in the results because this symptom was absent in nearly all of the patients.

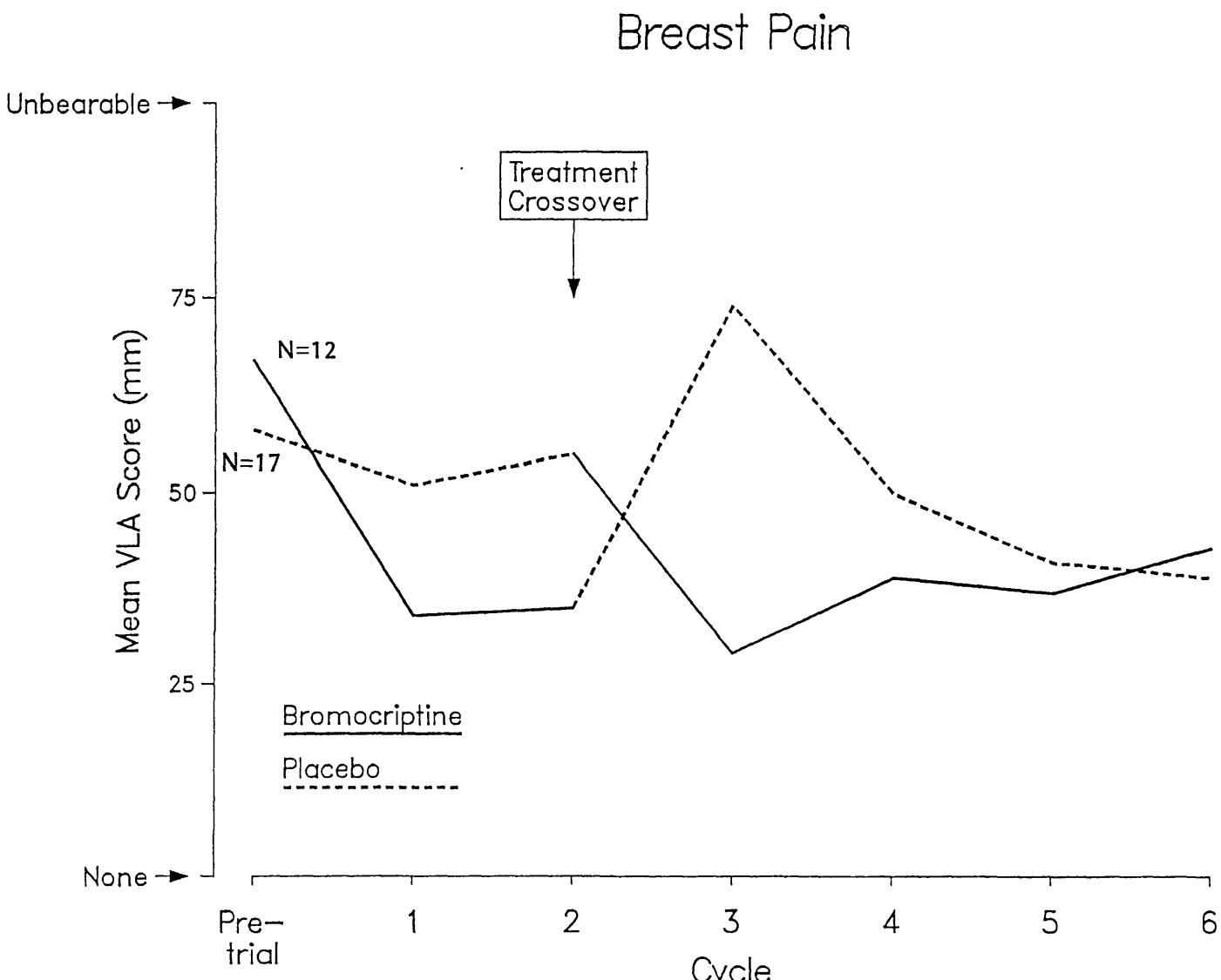


Figure 3.4 The mean VLA scores for breast pain in the 2 groups in the bromocriptine trial

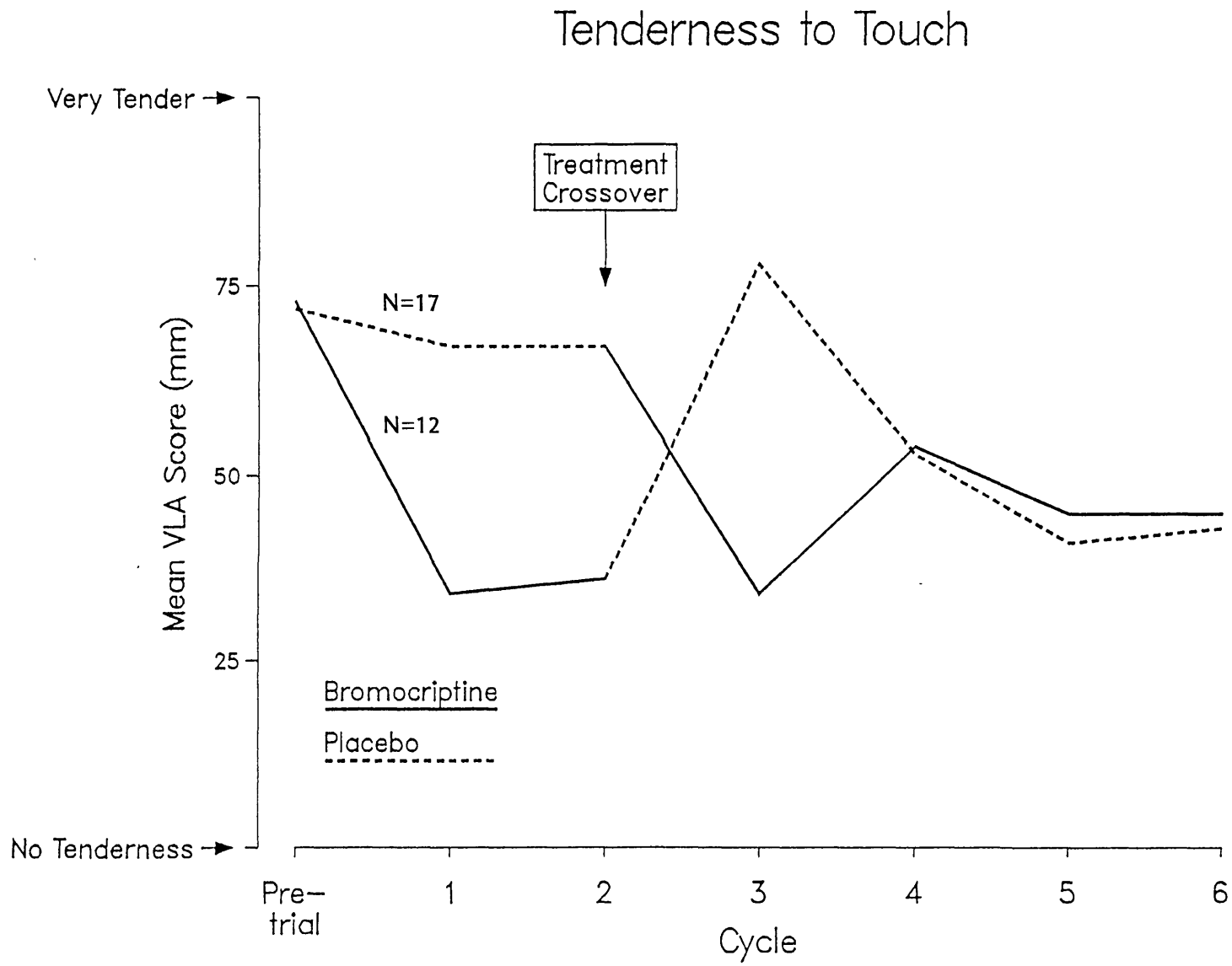


Figure 3.5 The mean VLA scores for breast tenderness in the 2 groups in the bromocriptine trial

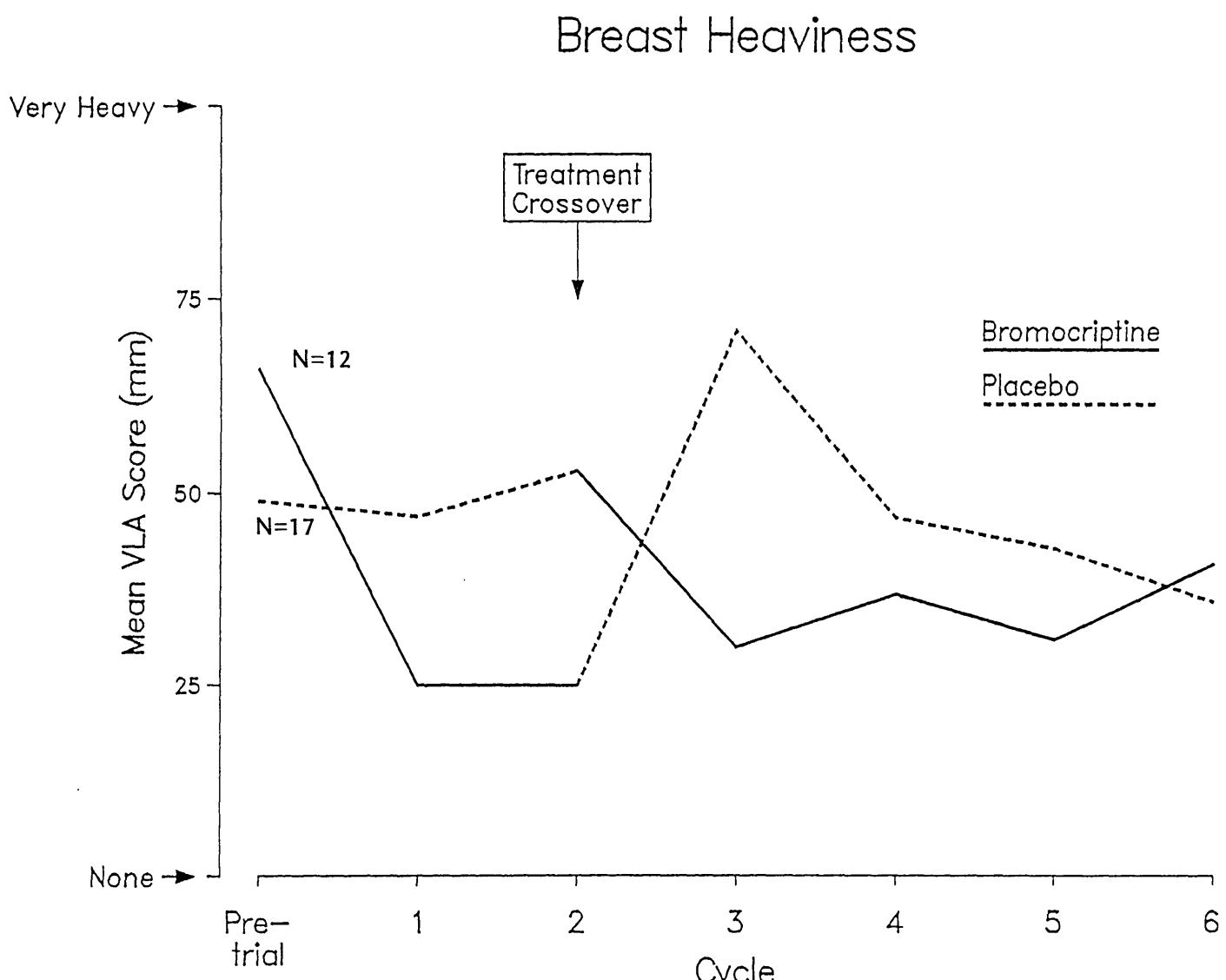


Figure 3.6 The mean VLA scores for breast heaviness in the 2 groups in the bromocriptine trial

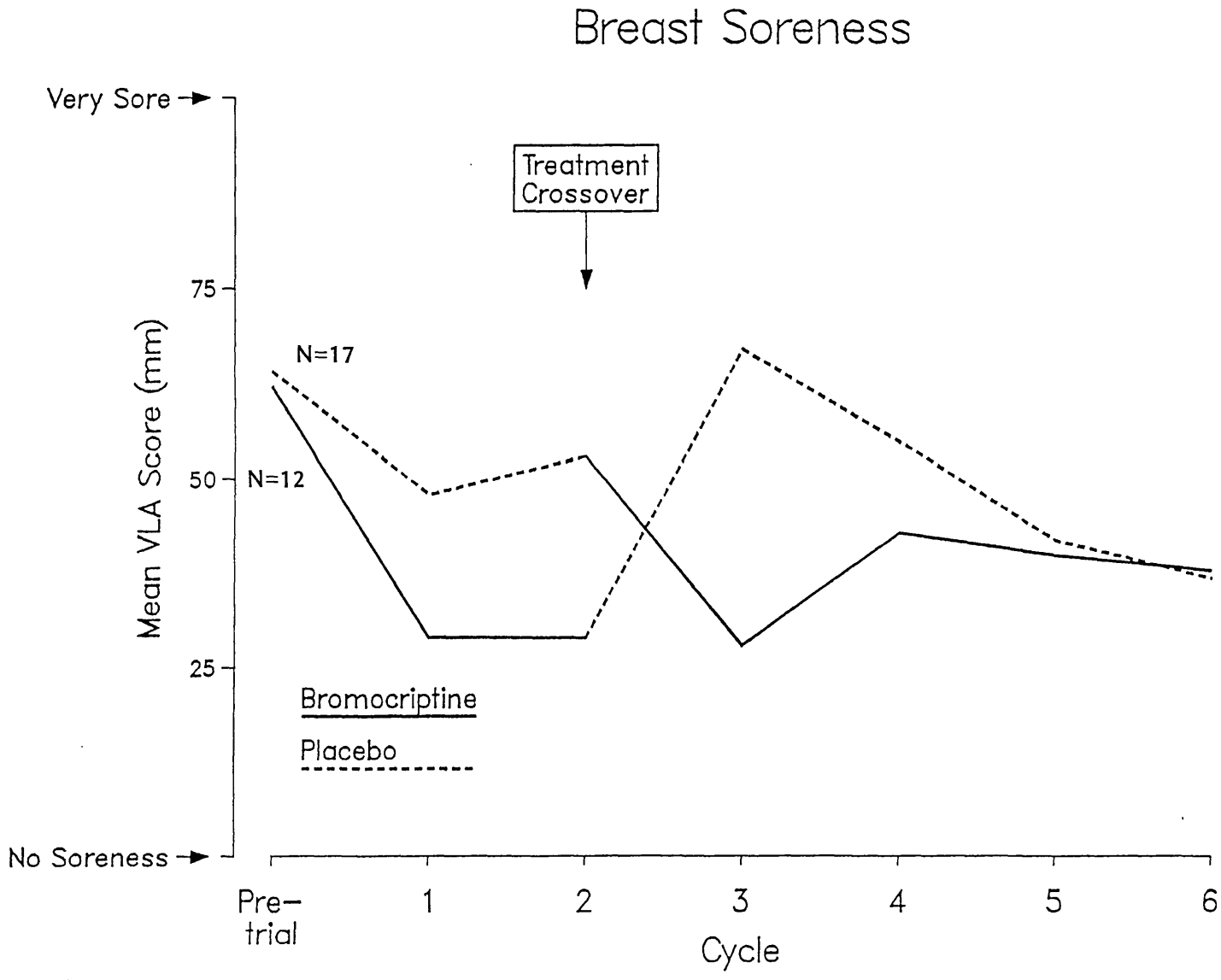


Figure 3.7 The mean VLA scores for breast soreness in the 2 groups in the bromocriptine trial

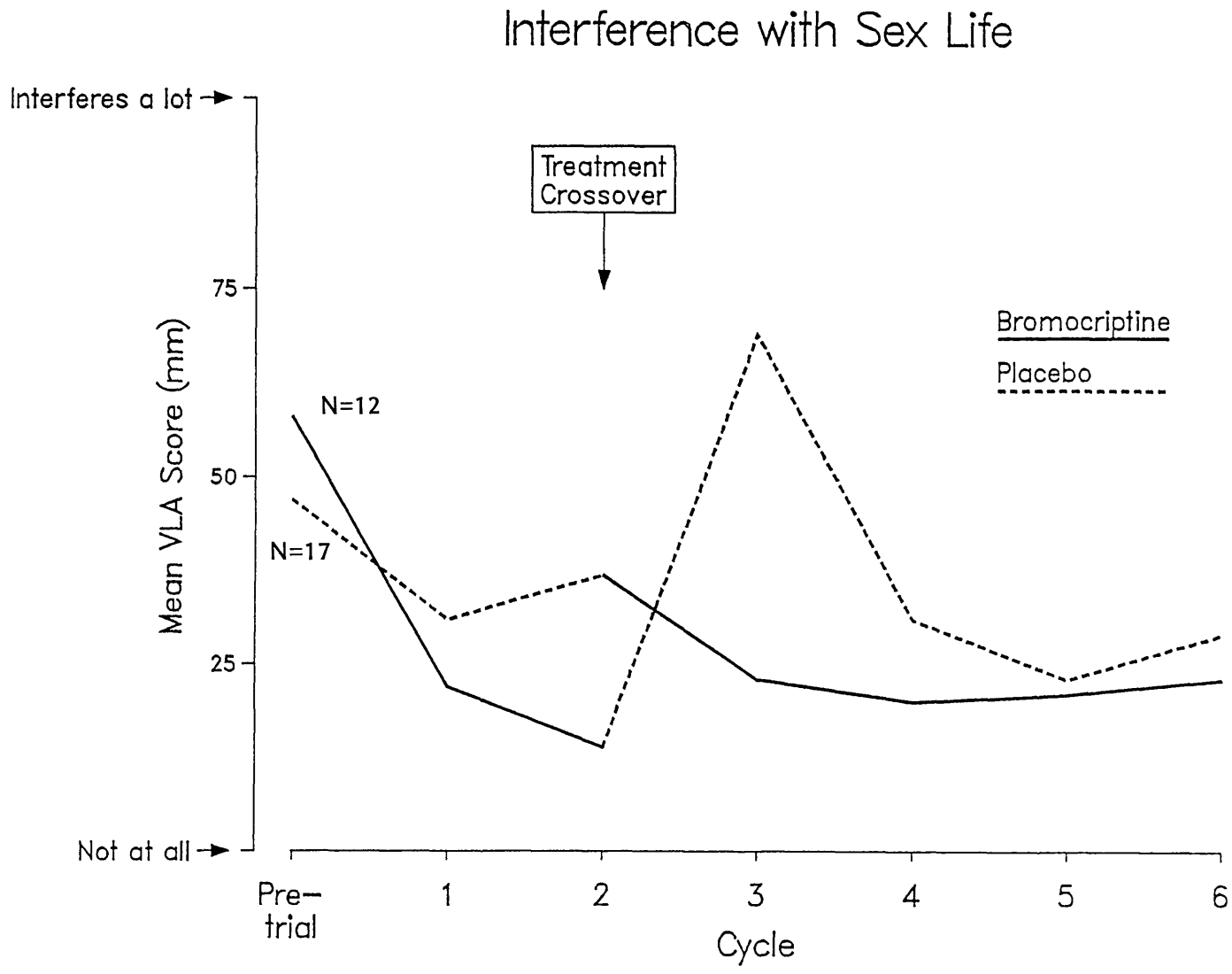


Figure 3.8 The mean VLA scores for interference with sex life in the 2 groups in the bromocriptine trial



Cyclical Mastalgia - VLA results for breast symptoms

Two principal analyses of the VLA data were performed using the Wilcoxon's matched pairs signed ranks test. First the data from patients who completed the whole 6 months of the trial were compared at pretrial, at the end of the first treatment period and at the end of the second treatment period. The mean scores throughout each treatment were also compared. Secondly an analysis was performed using all the randomised patients whether they completed the whole course or not (intention to treat basis). In this second analysis only the pretrial and the end of first treatment score could be compared, as the dropouts had no second treatment score to use. Two patients (No 5C and 38C) did not even return the first months VLA form and therefore only pretrial figures were available. These 2 were dropped from the analysis as they had no 2nd score to make a pair of data measurements for the Wilcoxon test.

Twenty nine cyclical patients with full data for 6 months of the trial were first analysed for efficacy of treatment. Twelve patients had been randomised to the bromocriptine -> placebo group and seventeen to the placebo -> bromocriptine group. The mean scores throughout the trial are plotted for illustration as graphs in Figures 3.4 (Pain), 3.5 (Tenderness), 3.6 (Heaviness), 3.7 (Soreness) and 3.8 (Interference with sex life). The statistical tests corresponding to these graphs are shown in tables 3.4, 3.5 and 3.6 which give the rank sum values of the Wilcoxon's signed rank tests. Figure

3.4 shows that there was a mild placebo effect in the first cycle in the group randomised to take placebo as the first therapy, but the score then rises, while the first cycle of bromocriptine shows a decline in breast pain which is maintained up to cycle 2 when the crossover occurs (Fig 3.4). Similar patterns are seen in all of the breast symptoms, although the placebo treatment scores that follow bromocriptine therapy in the bromocriptine -> placebo group are very variable (Figs 3.4 to 3.8).

The results of the statistical tests showed that the scores for breast pain, tenderness, heaviness, soreness and disturbance of sex life at the end of bromocriptine treatment were significantly lower ($p < 0.05$) than the pretrial scores (Table 3.4). Table 3.4 also gives the rank sums for the comparison between the score at the end of the bromocriptine period and the score at the end of the placebo period, in the patients who were randomised to bromocriptine as the first therapy. There were no significant differences between the 2 scores.

Similarly in the group randomised to take placebo first, the 2 comparisons of pretrial score versus end of placebo score, and end of placebo versus end of bromocriptine score were made and the rank sums are shown in Table 3.5. At the end of the placebo period the scores for breast pain, heaviness, tenderness, soreness and effect on sex life were not significantly better than pretrial levels, suggesting no significant effect of the placebo on breast symptoms. When the scores at the end of the

Table 3.4 VLA Analyses of cyclical group - Bromocriptine -> Placebo

	Rank Sums*	
	Pretrial v end Bromocriptine	End bromocriptine v end placebo
Breast pain	-13+65[a]	-30.5+47.5
Breast heaviness	-9+69[a]	-26+40
Breast tenderness	-7.5+68.5[a]	-27+39
Breast soreness	-9.5+56.5[b]	-11+44
Affects sex life	-8+47[c]	-3+20

* Wilcoxon's matched pairs signed ranks test

a $p < 0.05$ pretrial v bromocriptine for 12 pairs

b $p < 0.05$ pretrial v bromocriptine for 11 pairs

c $p < 0.05$ pretrial v bromocriptine for 10 pairs

bromocriptine treatment period were compared with those at the end of the preceeding placebo period in the placebo -> bromocriptine group, only the effect on sex life was significantly improved by bromocriptine (Table 3.5, rank sum - 3 + 33, $p < 0.05$ for 8 pairs). The smaller number of pairs in this comparison occurred because several patients reported no change while others were not sexually active.

As the single score taken at the end of each treatment period varied in comparison with other scores during the treatment , the analyses were repeated using the mean value for the bromocriptine and placebo periods for each individual. These results are shown in Table 3.6 for both the bromocriptine -> placebo and the placebo -> bromocriptine groups. These comparisons show a significant increase in breast heaviness in the placebo period following bromocriptine therapy, suggesting a return of symptoms. When bromocriptine treatment followed placebo, the level of breast tenderness and effect on sex life were both significantly improved compared with the placebo period when mean scores were used in the analysis (Table 3.6 $p < 0.01$ Rank Sums -15+121 for tenderness and -2+53 for sex life). Thus the analyses which use all of the VLA scores showed a greater effect of bromocriptine than the individual end of treatment scores but placebo effects remain statistically insignificant.

The second analysis was based on the 'intention to treat' principle, and all the randomised patients were included whether they dropped out or not subsequently. As most of

the dropouts had occurred in the bromocriptine to placebo group (table 3.3), the addition of these patients increased the bromocriptine -> placebo group from 12 to 17 and the placebo -> bromocriptine group from 17 to 18 . As 6 of the 9 cyclical dropouts occurred in the first treatment period, data was only available for the comparison between pretrial scores and the end of the first treatment period. If the patient dropped out in the first month, that score was taken for the comparison. The rank sums for the 'intention to treat' analyses are shown in table 3.7. There were no significant changes in the breast symptoms after 2 months placebo treatment in the patients randomised to placebo as the first therapy. In the group randomised to bromocriptine as the first therapy, all the breast symptoms were significantly reduced after 2 months treatment with bromocriptine, with heaviness, soreness and effect on sex life being highly significantly reduced. When the rank sums using all the randomised patients (table 3.7) are compared with those for the patients who completed the full trial (tables 3.4 and 3.5), it can be seen that the placebo treatment remains ineffective,but the improvements on bromocriptine tend to become more significant ($p < 0.05$ to $p < 0.01$ for most symptoms). This suggests that although more patients dropped out on bromocriptine therapy, they were experiencing relief of breast symptoms at the time.

Treatment interactions were checked by Koch's non-parametric test using the Mann-Whitney-Wilcoxon test (Koch 1972, Fleiss 1986), and no significant differences were found for the breast symptoms as measured by the VLA ($X2=1.26$ for

Table 3.5 VLA Analyses of cyclical group - Placebo -> Bromocriptine

	Rank Sums*	
	Pretrial v end placebo	End placebo v bromocriptine
Breast pain	-53.5+52.5	-41+79
Breast heaviness	-53.5+51.5	-55.5+78.5
Breast tenderness	-36+69	-38+115
Breast soreness	-41+112	-48+88
Affects sex life	-23+32	-3+33[a]

* Wilcoxon's matched pairs signed ranks test

a $p < 0.05$ end of placebo v end of bromocriptine for 8 pairs. (in favour of bromocriptine)

Table 3.6 VLA Analyses of Cyclical Group - mean treatment values

	Rank Sums of mean bromocriptine and placebo scores*	
	Bromocriptine-> Placebo	Placebo -> Bromocriptine
Breast Pain	-20+58	-37+116
Breast Heaviness	-10+56[a]	-44+109
Breast Tenderness	-17+61	-15+121[b]
Breast Soreness	-19+47	-44+109
Affects Sex Life	-5+23	-2+53[c]

* Wilcoxon's matched pairs signed rank test using the individual mean value of all placebo scores and all bromocriptine scores.

a $p < 0.05$ in favour of bromocriptine for 11 pairs

b $p < 0.01$ in favour or bromocriptine for 16 pairs

c $p < 0.01$ in favour of bromocriptine for 10 pairs

Table 3.7 VLA Analyses for all randomised cyclical patients'Breast Symptoms ('Intention to Treat' analysis)

	Rank Sums*	
	Placebo -> Bromocriptine N=18 Pretrial v end of placebo	Bromocriptine -> placebo N=17 Pretrial v end of bromocriptine
Breast pain	-53.5+66.5	-30+123[a]
Breast heaviness	-56.5+63.5	-15.5+120.5[b]
Breast tenderness	-37+83	-18+135[a]
Breast soreness	-51+120	-12.5+107.5[c]
Affects sex life	-26+40	-8.5+82.5[d]

a $p < 0.05$ (17 pairs)

b $p < 0.01$ (16 pairs)

c $p < 0.01$ (15 pairs)

d $p < 0.01$ (13 pairs)

*All tests by Wilcoxon's matched pairs signed ranks test

tenderness, X2=0.66 for breast pain).

Cyclical mastalgia - VLA results for general symptoms

Analysis of the VLA scores for general symptoms in the cyclical patients (Tables 3.8 and 3.9) showed that the symptoms of irritability and depression were significantly improved on bromocriptine in the patients who took this drug as the first therapy (Table 3.8). Irritability was improved most (Rank Sum -1+54, $p<0.01$ Wilcoxon's test), whilst depression just reached significance (Rank Sum -10+56, $p=0.05$ Wilcoxon's test). Placebo failed to improve any of the general symptoms when it was taken as the first therapy (Table 3.9). Neither bromocriptine nor placebo produced any improvement in general symptoms when they were given as the second treatment (Tables 3.8 and 3.9).

Non-Cyclical Mastalgia.

The VLA results in this group were different from those in the cyclical patients (Tables 3.10 and 3.11). There was only one significant difference between placebo scores and bromocriptine scores in either the breast or general symptoms, whether the analyses were performed using individual or mean scores. This was for the symptom of tenderness which reached a significance of $p < 0.01$ in favour of bromocriptine (Table 3.10. Rank Sum +44-1, 9 pairs). The number of patients is small and thus the analysis was performed on the patients as a group irrespective of treatment order, as the Wilcoxon's test is weak

Table 3.8 VLA Analyses of Cyclical Group - Bromocriptine -> Placebo

General Symptoms

	Rank Sums*	
	Pretrial v end bromocriptine	End bromocriptine v end of placebo
Headache	-14+41	-18.5+26.5
Feeling irritable	-1+54[a]	-32+23
Feeling depressed	-10+56[b]	-20+35
Bloated feeling	-18+48	-25+30
Well being	-19+59	-45+33
Tiredness	-21+34	-14+31

*Wilcoxon's matched pairs signed ranks test

a $p < 0.01$ pretrial v bromocriptine for 10 pairs

b $p = 0.05$ pretrial v bromocriptine for 11 pairs

Table 3.9 VLA Analyses of Cyclical Group - Placebo -> Bromocriptine

General Symptoms

	Rank Sums*	
	Pretrial v end placebo	End of placebo v end bromocriptine
Headache	-25+41	-58+46
Feeling irritable	-35+101	-26+79
Feeling depressed	-62+90	-37.5+82.5
Bloated feeling	-72+54	-44+76
Well being	-31.5+73.5	-51+69
Tiredness	-38.5+52.5	-23+68

*Wilcoxon's matched pairs signed ranks test

Table 3.10 VLA Analyses Breast Symptoms Non-Cyclical Group

All cycles irrespective of treatment order

	Rank sums*
	End bromocriptine v end placebo
Breast Pain	+21-24
Breast Tenderness	+44-1[a]
Breast Heaviness	+14-12
Breast Soreness	+14-22
Affects Sex Life	+9-4

* Wilcoxon's matched pairs signed ranks test for 9 pairs

a $p < 0.01$ end of bromocriptine v end of placebo for 9 pairs (in favour of bromocriptine)

when less than 6 pairs of data are available. But, the general lack of response in the non-cyclical patients fits in well with the higher dropout rate observed in this group. The Rank Sum for tiredness was not calculated as there were only 3 pairs with complete data for this symptom.

(c) Clinician's assessment response.

Cyclical Mastalgia

The clinician scored the response on a scale from 1 to 3, and the mean and standard error for the clinician's rating of tenderness and nodularity in the cyclical group are shown in table 3.12. The pre trial mean scores (2.52) show a high level of tenderness and nodularity, and correlate well with the pre trial VLA scores in this group of patients (figs 3.4 to 3.8). The rank sums show a statistically significant improvement in both tenderness and nodularity on bromocriptine therapy in the patients who took this drug as first therapy (T = -0+28 and -0+45 $p<0.01$, Wilcoxon's matched pairs signed ranks test). In the placebo -> bromocriptine group there was no significant improvement of either tenderness or nodularity while on placebo therapy. When bromocriptine followed placebo, it produced a significant reduction in nodularity (T = -0+21 $p<0.05$), whereas placebo treatment after bromocriptine therapy produced no significant improvement. Similarly, when bromocriptine therapy followed placebo, the score for 'treatment helping' was shown to be significantly improved compared with placebo (T = -11+67 $p<0.05$).

Table 3.11 VLA Analyses General Symptoms Non Cyclical Group

All cycles irrespective of treatment order

	Rank Sums*
	End bromocriptine v end placebo
Headache	+ 19-9
Feeling irritable	+ 8.5-19.5
Feeling depressed	+ 10-11
Bloated feeling	+ 14-14
Well being	+ 22.5-13.5

*Wilcoxon's matched pairs signed ranks test

No significant differences

Table 3.12 Bromocriptine Trial - Clinician's Assessment

Cyclical Group

	Mean ± SE (All scores)	
	Tenderness	Nodularity
Pre Trial	2.52±0.10	2.52±0.10
Placebo	1.8±0.08	1.94±0.06
Bromocriptine	1.41±0.06	1.68±0.06

RANK SUMS

	TENDERNESS	NODULARITY	RESPONSE TO TREATMENT
Placebo -> Bromocriptine			
Pretrial v. end placebo	-7+38	-8+24	N/A
End placebo v. end bromocriptine	-7+29	-0+21[a]	-11+67[b]
Bromocriptine -> Placebo			
Pretrial v. end bromocriptine	-0+28[c]	-0+45[d]	N/A
End bromocriptine v. end placebo	-6+15	-3.5+24.5	-7+21

a $p < 0.05$ 6 pairs

b $p < 0.05$ 12 pairs

c $p < 0.05$ 7 pairs

d $p < 0.01$ 9 pairs

* Wilcoxon's matched pairs signed ranks test

Non-Cyclical Mastalgia.

The scores for tenderness and nodularity in the non-cyclical group are shown in table 3.13. As in the cyclical group a small placebo effect is discernible, but the bromocriptine scores are not significantly better than those on placebo when compared by Wilcoxon's matched pairs signed ranks test. It appears that the patients with non-cyclical pain had lower pretrial scores for tenderness and nodularity, but this again fits with the profile of this group given in Fig 2.3 (Chapter 2) where it was noted that nodularity is uncommon.

(d) Serum Prolactin.

The results of the 37 patients who had pretrial prolactin levels estimated are shown in Fig 3.9. The upper limit of normal for the assay used by the Tenovus Institute was 35 mU/100ml and only 4 patients had values above this upper limit of normal. These 4 patients with mildly elevated prolactin levels had normal skull X-rays with no evidence of pituitary tumour. The elevation in these cases was only just above the upper limit of normal and well below the level usually seen in true hyperprolactinaemic patients. The mean levels of prolactin in the groups was 23.1 $\pm$2.4 mU (mean $\pm$ se) in the cyclical group and 20.6 $\pm$ 2.9 mU in the non-cyclical group, and these figures are not significantly different. The effects of placebo and bromocriptine therapy on these prolactin levels are shown in table 3.14. Bromocriptine produced a statistically significant

Table 3.13 Bromocriptine Trial - Clinician's Assessment

Non Cyclical Group

	Mean ± SE	
	Tenderness	Nodularity
Pre trial	2.01±0.09	1.66±0.21
Placebo	1.97±0.12	1.48±0.10
Bromocriptine	1.68±0.13	1.34±0.08

Wilcoxon's signed ranks test - No significant differences

Table 3.14 Prolactin levels in the bromocriptine trial

	(mU/100 ml Mean ± SE)		
	Pretrial	Placebo	Bromocriptine
Cyclical Mastalgia N=29	23.1±2.4	22.8±2.3	14.8±1.9*
Non Cyclical Mastalgia N=9	20.6±2.9	17.8±2.4	16.7±4.2

* $p < 0.05$ Student's t test v pretrial or placebo

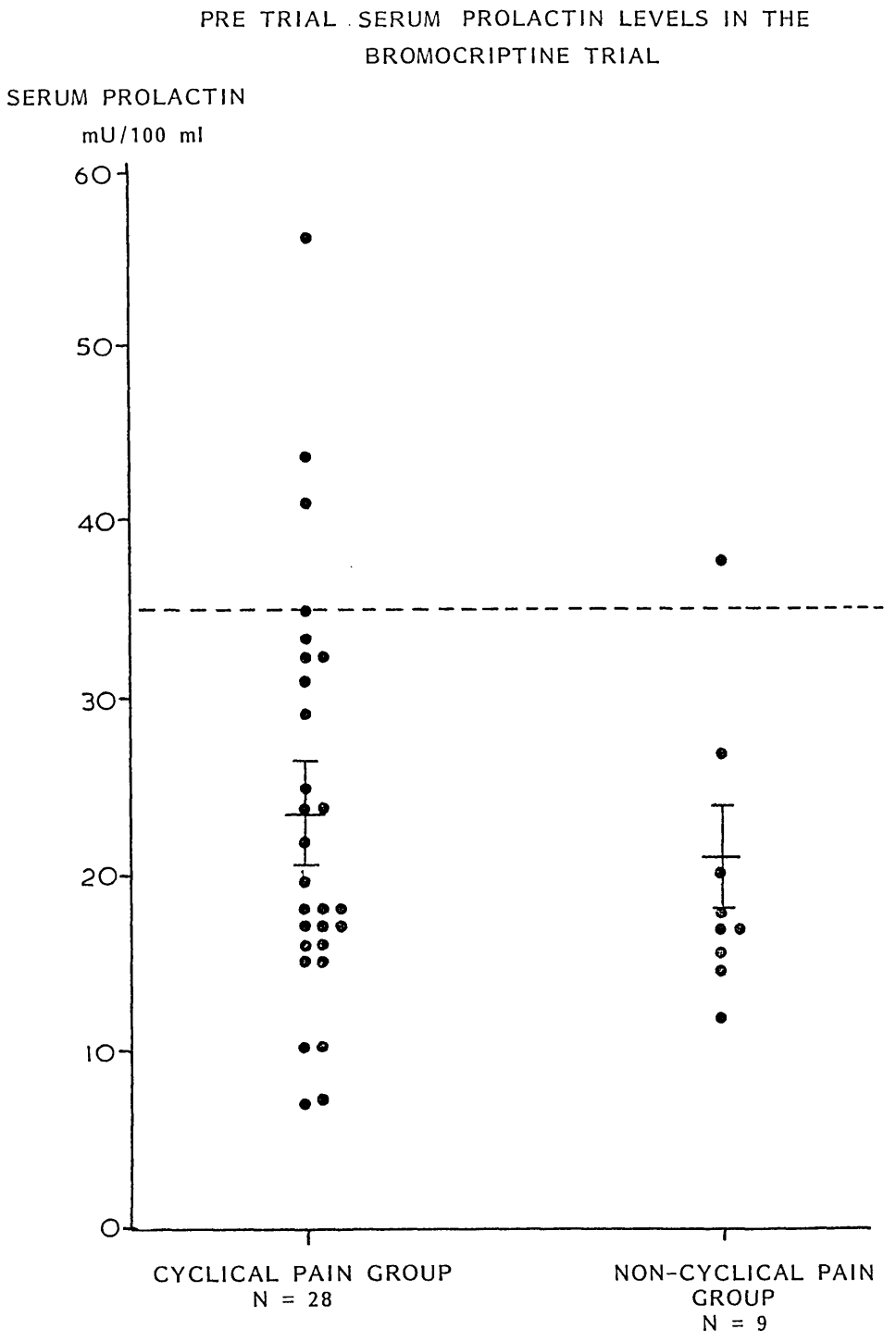


Figure 3.9 Pretrial serum prolactin values in the cyclical and non-cyclical patients in the bromocriptine trial. The error bars indicate 1 standard error. The dotted line indicates the upper limit of normal for the assay.

depression of prolactin in the cyclical patients but an insignificant effect in the non-cyclical group. However the numbers in the non-cyclical group were much smaller with only 9 patients, and thus it is not surprising that the fall in prolactin levels has not reached statistical significance. All the previous experimental work with bromocriptine in humans would predict a fall in prolactin levels in these cases also.

As about half of the patients had been randomised to take bromocriptine initially and then crossed over to placebo, a comparison could be made between their prolactin levels on bromocriptine and subsequently on placebo. These results proved extremely interesting as a marked rebound of prolactin secretion on cessation of bromocriptine was noted in these patients (Fig 3.10). The rebound occurred within days of stopping the drug and continued for at least a month. The results shown in Fig 3.10 are the means of these patients, but as the standard errors are wide there was considerable variation in individual responses. A more detailed analysis of the early time course of the rebound is shown in Fig 3.11 where the levels were estimated at intervals of 2 - 3 days after stopping bromocriptine. Two of the five patients had a high rebound which seemed to last up to 24 days, whereas the other 3 patients had lower rebounds. These results may indicate that there are endocrinologically different sub-types present in this group of apparently homogeneous patients, albeit selected on the basis of simple symptom patterns.

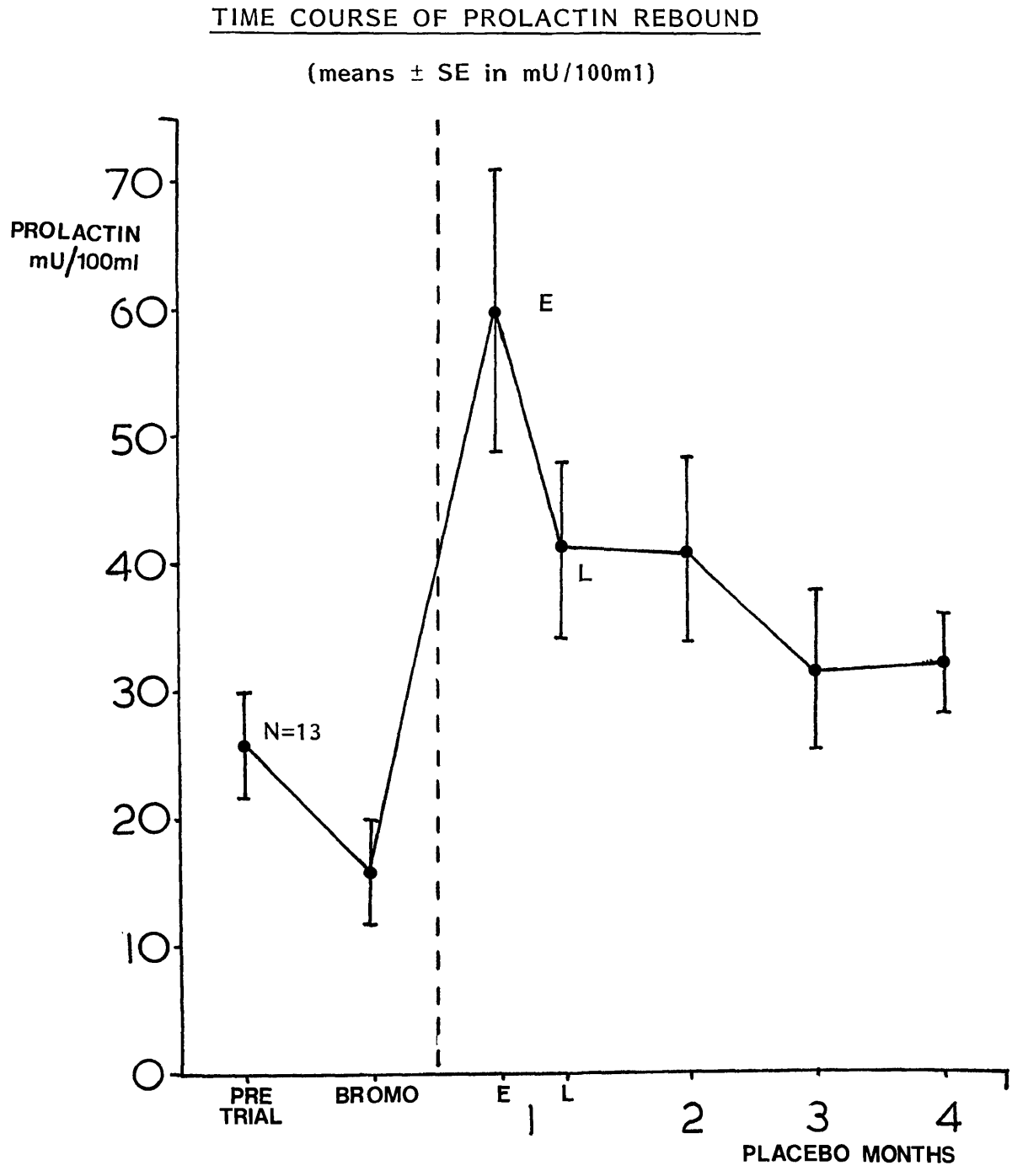


Figure 3.10 The mean serum prolactin level during the trial in the 13 patients who commenced bromocriptine and then crossed over to placebo (E = early placebo month 1, L = late placebo month 1, BROMO = on bromocriptine)

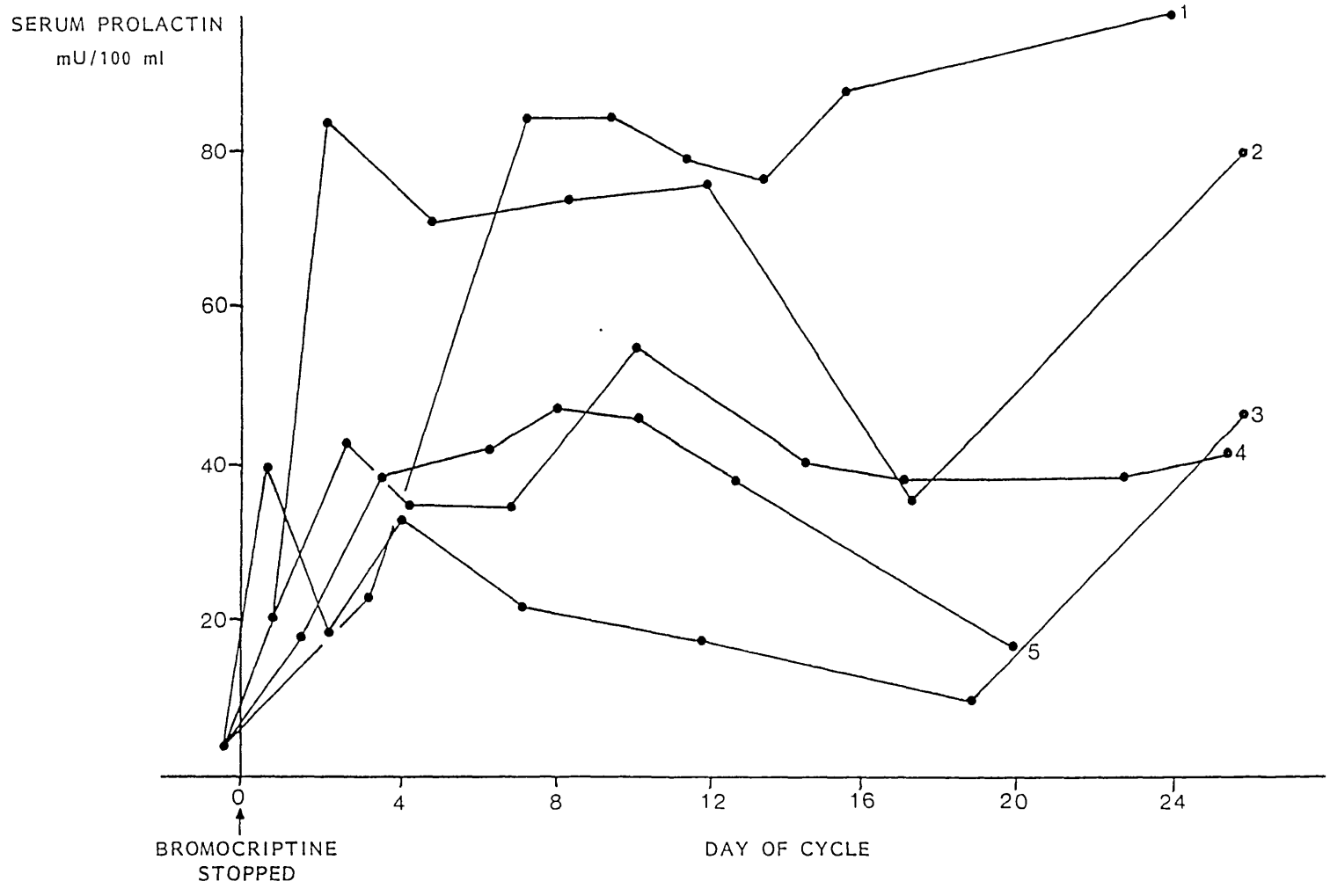


Figure 3.11 The time course of the prolactin rebound in 5 individual patients on stopping bromocriptine.

In order to see if there were any correlation between basal or bromocriptine suppressed prolactin levels, the results were plotted as the fall in prolactin levels in individual patients, against the improvement in symptoms (Fig 3.12) at each time point along the study for those patients who had prolactin levels estimated. There clearly is no correlation between the fall in prolactin levels and symptom improvement; if anything the negative correlation of the smallest fall in prolactin with the largest fall in symptoms seems to obtain.

(e) Side effects

Side effects were recorded on the VLA form where there was a blank 100mm line which the patient could fill in with a description of the alleged side effect (Appendix II). The commonest side effects recorded by the patients were nausea, headache and dizziness, which were experienced overall by one third of the 53 patients (Table 3.15) who were taking bromocriptine, but 8 patients also noted side effects while taking placebo. Nausea and headache were however significantly more common on bromocriptine, than on placebo (Chi square test $p < 0.05$).

This high incidence of side effects was seen despite the incremental dose regimen used (as detailed in table 3.1) and the instruction given to take the tablets with food. The impact of the side effects can be seen in the dropout patients who had a withdrawal rate of 15% in the cyclical group and 40% in the

non-cyclical group (Table 3.3). The patients who dropped out experienced the typical side effects of nausea, headache and faintness.

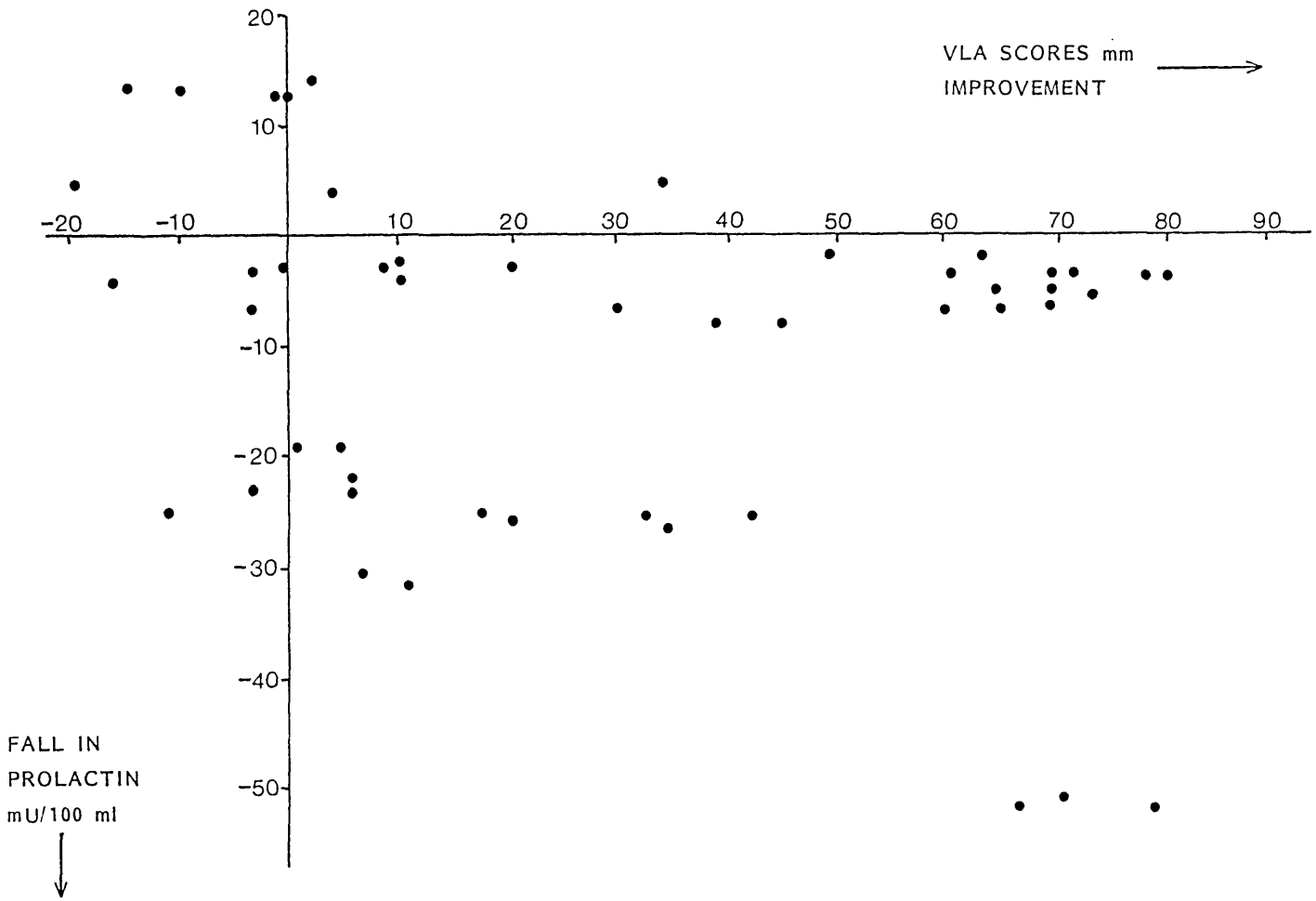


Figure 3.12 The fall in prolactin levels on bromocriptine plotted against the improvement in the VLA scores on the drug. There was no correlation between the 2 parameters.

Table 3.15 SIDE EFFECTS IN THE BROMOCRIPTINE TRIAL
ALL PATIENTS N=53

	ON BROMOCRIPTINE	ON PLACEBO
NAUSEA	13 (25)*	4 (8)
HEADACHE	10 (19)*	2 (4)
DIZZINESS	4 (8)	2 (4)
OVERALL SIDE EFFECTS	17 (32)	9 (17)

* $p < 0.05$ versus placebo X2 test

Figures in parentheses are percentages

3.4 DISCUSSION

The results of this controlled study demonstrated the beneficial effects of bromocriptine in patients with cyclical mastalgia,but showed limited effect in those patients with non-cyclical mastalgia. There could be several explanations for this difference in efficacy between the two groups. The non-cyclical group include patients who are post-menopausal and thus would be less likely to have an endocrine basis for their symptoms, unlike the cyclical group whose symptoms appear to be directly related to the menstrual cycle. The non-cyclical patients were a smaller group and thus were less likely to demonstrate statistical differences, especially as the data had to be pooled to analyse it with the Wilcoxon's test. It is possible that non-cyclical pain is a truly non-endocrine problem, and this has been supported by some recent work on dynamic pituitary testing (Kumar et al 1984 a+b, 1985) showing a normal TRH response in non-cyclical patients but an enhanced response in cyclical patients, which correlates with subsequent therapeutic response (Kumar et al 1985). The differential therapeutic response had not been noted in the early papers on bromocriptine, but case definition was poor in most studies, and no attempts were made to differentiate between pain patterns or stratify them in trial structures. This being so, it is likely that an unselected series of mastalgia patients would consist mainly of the cyclical pattern, as Preece et al have shown that 75% of mastalgia cases are cyclical in type (Preece et al 1976). Thus previous studies of bromocriptine in mastalgia would have

tended to consist mainly of cyclical cases who would be expected to respond to bromocriptine as demonstrated in this trial. Also the previous studies consisted of very small numbers and would not be able to show any differences between sub-groups. The finding of differential therapeutic response and prolactin response has allowed study of several new areas which have improved our understanding of the problem of mastalgia. The findings of this double blind study have now been reproduced by other controlled studies. Hinton et al using a double blind format found that bromocriptine produced significant decreases in breast pain, heaviness and tenderness measured on linear analogue scores (Hinton et al 1986a). Blichert-Toft et al also used a double blind crossover format in 10 women with mastalgia and showed total relief in 8 and partial relief in 2 on bromocriptine, whereas placebo only gave relief in 1 out of the 10 patients (Blichert-Toft et al 1979). Durning and Sellwood treated 24 patients with cyclical mastalgia in a double blind crossover trial, and showed significant improvement in breast pain, tenderness and engorgement on bromocriptine (Durning and Sellwood 1982). Recent controlled studies in the premenstrual tension syndrome have confirmed the efficacy of bromocriptine in reducing breast symptoms (Ylostalo et al 1981, Kullander and Svanberg 1979).

This study has also demonstrated the validity of the VLA system for the first time, in the setting of a trial of therapy for breast pain. Previous work had concentrated on measurement of feelings (Aitken 1969) rather than physical symptoms, although

VLA techniques had been used in the assessment of the chronic pain of rheumatoid arthritis (Huskisson 1974) and post anaesthetic pain (Revill et al 1976). In this study the VLA scores correlated well with the clinician's assessment of response to therapy although there was no obvious correlation with prolactin levels. The patients found the VLA forms easy to complete and there was a high rate of compliance with this technique. Since these forms are completed by the patient, they can be mailed to her home and returned to the investigator, thus making it easier for the patient as fewer hospital attendances are required. The main disadvantage of the VLA is that no comparison across groups can be made as the score of one patient is not directly comparable with any other patient's score. However the system can be used to compare the same patient's score at different time points during the trial. In this study the differences between the score on therapy and off therapy were assessed by the use of Wilcoxon's signed ranks test as this non-parametric test takes into account both the magnitude and direction of any difference, as obviously a patient with a VLA score of 50 mm could either get worse or improve. In this test no change in score between time points removes that score from the ranks, and does not affect the result (Appendix I). This allowed the analysis of all the patients who had completed a course of both bromocriptine and placebo. None of the previously reported work on bromocriptine had used a VLA system of assessment, most relying on a simple scale assessment by the clinician who was not blind to the treatment. The problems of a simple scale have been noted in chapter 2, and it is obvious that an open study of a new

and unique drug would induce an increased level of expectation in the investigator which could be passed on to the patient. This effect coupled with the lack of placebo control means that the early studies of bromocriptine in breast pain were not interpretable. The double blind, placebo structure of the study in this chapter, together with careful assessment and selection of patients, makes this the first properly controlled study for the effects of bromocriptine in mastalgia.

Another interesting area of difference between this study and previous work is that it has demonstrated that the side effects of bromocriptine are more common than previously reported (Mansel 1981). The technique of "volunteered" side effects used in this trial does increase the overall level of side effects reported, as the patient's attention is drawn to this issue. The VLA form was used to allow side effects to be recorded without discussion with the clinician as this would hazard the double blindness of the study. The previous open studies with small numbers of patients, reported few side effects and no dropouts (Schulz et al 1975, Martin-Comin et al 1976, Mussa and Dogliotti 1979), thus suggesting the drug would be well tolerated in most patients. There was good evidence that side effects were few in hyperprolactinaemic patients and pregnant women who are, of course, transiently hyperprolactinaemic. When the drug was used to suppress lactation few side effects were seen (Walker et al 1975). However this present study shows a high incidence of side effects in women who are normoprolactinaemic and this experience has been confirmed by more recent controlled studies showing an

increasing incidence of side effects (Durning and Sellwood 1982, Hinton et al 1986a, Blichert-Toft et al 1979). Durning and Sellwood reported a 30% incidence of severe and persistent nausea, headache and dizziness (Durning and Sellwood 1982) which is similar to the results reported here. The official advice given to patients is that they should take the first few doses at night with food (Parlodel Data Sheet 1986), but despite these precautions there are some women who cannot tolerate the drug at any dose. The symptoms of postural hypotension and dizziness can be severe enough to confine the patient to her bed, as happened to one of the patients in this trial. As a result of these findings the data sheet has been modified to recommend a gradual increase in dosage, and the Sandoz Pharmaceutical Company are investigating a new injectable depot form of bromocriptine in the hope of reducing side effects (Peters et al 1986). It does appear that this slow release formula gives rise to fewer side effects than the parent compound (Lancanjran et al 1986).

This study showed there was no relationship between basal prolactin levels or the extent of suppression, and the clinical outcome of bromocriptine therapy. Clearly there is no simple relationship between serum prolactin changes and symptom changes in the breast. There are a large number of ways in which prolactin could affect hormone expression at the target tissue and many of the hormones themselves have complicated inter-relationships. Thus it is not very surprising that a simple correlation cannot be demonstrated, although such a relationship does hold for prolactin suppression and tumour shrinkage in the

case of prolactinomas treated with bromocriptine (Sobrinho et al 1981). Recent work emanating from the rebound data seen in this bromocriptine trial has shown that cyclical and non-cyclical patients have a significantly different response of prolactin secretion to a thyrotropin releasing hormone test (TRH), or domperidone test (Kumar et al 1984 a+b).

Further, it has been shown that the peak response of prolactin to TRH or domperidone performed pre-therapy predicts the subsequent response to hormone therapy in cyclical mastalgia patients (Kumar et al 1985). This finding of an enhanced TRH response has now been confirmed by several other groups of investigators (Dogliotti et al 1985, Peters et al 1981, Watt-Boolsen et al 1985).

In summary, this trial demonstrated the efficacy of bromocriptine in cyclical mastalgia, the failure of response of non-cyclical mastalgia and the high rate of side effects of bromocriptine. The data on the variable rebound prolactin levels have led to the evolution of the TRH test as a useful test for predicting response to hormone therapy in mastalgia patients. The validity of this approach is currently being tested in a European multicentre trial of bromocriptine in cyclical mastalgia.

CHAPTER FOUR

THE DANAZOL TRIAL

4.1. INTRODUCTION

4.2. PATIENTS AND TRIAL METHODS

4.3. RESULTS

4.4. DISCUSSION

4.1. INTRODUCTION

Danazol was synthesised in 1963 at the Sterling-Winthrop Research Institute and is derived from ethisterone. It is a steroid with an isoxazole group on the A ring of the steroid nucleus, which is ethisterone for danazol (fig 4.1). Danazol reaches a peak in human plasma at 2-3 hours after ingestion of a single 400 mgs dose (Davison et al 1976). The plasma half-life is about 4 1/2 hours and steady state plasma levels are reached after 7 days dosing at 200 mgs daily. The mode of action of the drug is not clear and it may work by several different mechanisms (Barbieri and Ryan 1981). The 4 most likely actions are:-

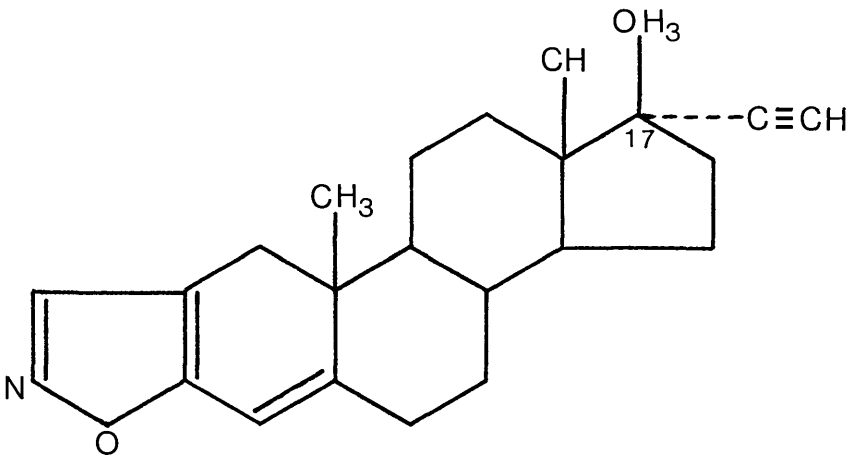
(a) direct inhibition of gonadotrophin releasing hormone secretion and/or gonadotrophin secretion,

(b) direct inhibition of multiple enzymes of steroidogenesis,

(c) interaction with intracellular androgen and progesterone receptors,

(d) alteration of endogenous steroid metabolism.

Several studies in menopausal women and primates demonstrate that danazol has an antigonadotrophic action (Franchimont and Cramilon 1977, Krey et al 1981). In normal men danazol at a dose of 200-600 mgs does not significantly affect luteinising hormone (LH) levels (Sherrins et al 1971) and in pre-menopausal women the basal levels of LH and follicle



Danazol (2, 3 is oxazol ethisteron)

Figure 4.1 The chemical structure of danazol

stimulating hormone (FSH) are not affected and a normal response to luteinising hormone releasing hormone (LH-RH) occurs (Andrews and Wentz 1975,Wood et al 1975,Lauerson and Wilson 1976). However, when doses of danazol ranging from 600-800 mgs are given, these always suppress the mid-cycle surges of LH and FSH, thus preventing ovulation (Greenblatt et al 1971). This suppression of the menstrual cycle includes amenorrhoea but with normal basal levels of LH, FSH, prolactin and oestrogen (Luciano et al 1981). The prevention of ovulation, of course, is reflected by low levels of progesterone normally seen in the follicular phase of the cycle. Evidence for a direct effect on the gonads comes from studies showing suppression of gonadal hormones in men (Sherrins et al 1971), children (Lee et al 1975) and pre-menopausal women (Wood et al 1975) in the absence of corresponding suppression of gonadotrophins. Danazol also inhibits progesterone production in vitro in human luteal cells (Henderson and Tsang 1980), and adrenal steroidogenesis in vitro in healthy pre-menopausal women (Stillman et al 1980).

Receptor interactions have been demonstrated in uterine tissue for oestradiol (Cook and Gibb 1980), progesterone (Tamya et al 1978, Jenkin 1980) and androgens (Chamness et al 1980). The drug could, of course, interact with the corresponding receptors wherever they may occur, and so effects in the hypothalamus, pituitary, ovary and the breast itself could result. It can be seen from this review that danazol has multiple actions, and its clinical effect could result from any one of these although it

seems unlikely that a single effect could explain all the observed actions.

Whatever the actual mechanisms involved, danazol does have predictable effects on the human menstrual cycle at oral doses of 600-800 mgs daily. Patients become amenorrhoeic within 4-8 weeks, the endometrium becomes atrophic and vaginal smears become hypo-oestrogenic. The mid-cycle surges of LH and FSH become blunted or are absent, but basal levels of FSH and LH are only slightly lowered (Greenblatt et al 1971). These changes produce a "medical castration" which completely reverses on stopping the drug (Wood et al 1975, Greenblatt et al 1974). The drug may also act as a weak androgen and is mildly anabolic. It is however devoid of oestrogenic or progestational activity by standard pharmacological tests. In humans there is no effect on dynamic adrenal function (ACTH test) although the levels of adrenal steroids other than cortisol may be altered (Barbieri et al 1980). Danazol does reduce the levels of thyroid binding globulin although no clinical effects of this change have been seen (Graham and Gambrell 1980). Danazol at 600 mgs per day has been shown to reduce T3 levels in mastalgia patients, although no change in the TSH response to TRH was demonstrated (Peters et al 1980).

The effects of the drug on serum prolactin are not clear. In Rhesus monkeys long term administration of danazol does not effect the serum levels of prolactin (Asch et al 1979). In

humans with BBD there are some reports of a reduction of serum prolactin on danazol at doses of 400 mgs (Greenblatt et al 1980) and 800 mgs daily (Bohnet et al 1981). Conversely, other studies in BBD patients (Montgomery et al 1979, Nezhat et al 1980, Buckle 1980, Peters et al 1980) and endometriosis (Floyd 1980, Guillebaud et al 1977) have failed to show any effect on serum prolactin. These observations are supported by the finding that danazol does not suppress lactation when given to lactating women (De Gezelle et al 1978), unlike bromocriptine which readily suppresses lactation. Peters did show that although the basal prolactin did not change on 600 mgs of danazol per day, the prolactin response to TRH was blunted by the drug (Peters et al 1980).

Although the first human studies were carried out in patients with endometriosis, Greenblatt et al in 1971 treated a number of cases of BBD (2 cases of virginal breast hypertrophy and 5 of cystic breast disease), and they noted an improvement in breast discomfort in 3 of the 5 cystic disease cases, and a decrease in breast size in both virginal hypertrophy cases (Greenblatt et al 1971). Further reports then appeared from Greenblatt's group describing the use of danazol in the treatment of "fibrocystic mastopathy", and reporting a substantial degree of success in relieving the symptoms of breast pain and nodularity (Asch and Greenblatt 1977, Aksu et al 1978, Greenblatt et al 1980, Greenblatt and Ben-Nun 1980, Nezhat et al 1980, Greenblatt et al 1982). The last paper described the treatment of 130 women and reported a 70% elimination of breast nodularity at daily doses of

100-400 mgs danazol (Greenblatt et al 1982). In this paper Greenblatt suggested that the reduction of nodularity in these BBD patients might reduce the need for breast biopsy in some women, a concept which may have some relevance in patients with very lumpy breasts. Nevertheless, the major problems of studies in breast pain and nodularity are highlighted in this series of papers from this unit - the selection of patients was not defined, assessment of response has not been described, and no controls of any sort have been used. Thus despite this impressive list of publications the role of danazol in symptomatic BBD has not been defined in a controlled study. The emphasis of the Greenblatt group has evolved from alleviation of breast pain to reduction in breast nodularity with a hypothetical reduction in hyperplasia in the breast epithelium, although there is no good evidence that danazol or any other agent can produce a regression in hyperplasia in cases of histologically proven hyperplasia.

Other open studies have been carried out in the USA but the poor definition of patients and lack of controls are repeated in all the studies. Brookshaw reviewed several multicentre studies in the USA involving 514 patients, and although a double blind study is mentioned in the methods, the results refer only to the whole group of patients pooled together (Brookshaw 1979). This paper did note the incidence of amenorrhoea to be 60-70% on 400 mgs daily and weight gain was recorded in 18% of the patients. An open study of 40 patients with chronic cystic mastitis by Lauerson and Wilson reported improvement in breast symptoms in

87% of patients at a dose of 400 mgs, but many of their patients were anovulatory as shown by the low pretrial progesterone levels (Lauerson and Wilson 1976). A further U.S. study of 30 patients with fibrocystic disease at 3 different dose levels showed complete relief of pain and tenderness in 18 out of the 23 patients who completed the study (Baker and Snedecor 1979). Again, weight gain was common with 6 of 23 patients complaining of this side effect. Humphrey and Estes treated 18 women with "fibrocystic disease" with either 400 or 100 mgs daily doses and reported a 75-100% response rate in an open study, and noted a 9% dropout rate. (Humphrey and Estes 1979). Blackmore reported open studies involving 260 patients in several centres, but the results of therapy appear to include only 112 patients and several different doses are quoted (Blackmore 1977). He makes the comment that results of double blind studies will be forthcoming, but in recent conversation with Dr. Blackmore (who heads the Sterling Research Institute in New York) it appears that the double blind studies referred to have not been completed and have not been published (Blackmore - personal communication 1982). Thus although the papers quoted appear to study large numbers of patients, these have been conducted by a large number of investigators and have no controls. Thus it was clear in 1978 that there was a need for a properly controlled study of danazol in patients with well defined breast pain.

4.2 PATIENTS AND TRIAL METHODS

(a) Patients.

For the danazol study only patients with cyclical mastalgia were selected for therapy, as the earlier bromocriptine trial had shown that non-cyclical patients were unresponsive to hormone manipulation. It was therefore considered unethical to admit these patients to a further hormone-based study against placebo, when it was suspected that they might be an unresponsive group. The protocol of the danazol study was submitted to the Division of Surgery Ethical Committee which approved the study. Patients were selected after referral from the Breast Clinic and the pain chart had shown a cyclical pattern of breast pain with total duration of pain of at least 1 year. Patients were over 18 and were not on the oral contraceptive pill. They were limited to a weight range of 40-91 Kgs, as previous studies had shown a greater anabolic effect of danazol in women of above average weight. Patients were excluded from the trial if they were suffering from current hepatic or renal disease, had any gynaecological disorder, had any known previous allergic reaction to danazol or were diabetic.

At review in the Mastalgia Clinic the mammogram was checked to make sure it was benign. If the patient satisfied the criteria for entry into the study, the trial was explained to her, including the placebo arm, and oral informed consent was

obtained.

Twenty eight patients satisfied the eligibility requirements and were entered into the study. Twenty two patients completed the full 6 months course of the study and there were 6 dropouts as detailed in the results section.

(b) Methods

The trial structure was a double blind, placebo-controlled, single crossover design lasting for 6 months as shown in fig. 4.2. Thus one group of patients were randomised to receive danazol for 3 months followed by 3 months placebo, and the second group placebo for 3 months followed by danazol for 3 months. Danazol capsules at 200 mgs twice daily for the first 6 patients and 100 mgs twice daily for the remaining patients were supplied pre-randomised in blocks, by Winthrop Pharmaceuticals Ltd., who also supplied matching pre-randomised placebo capsules. The differing doses were chosen to see if the side effect profile was different at the 2 doses, as previous work had suggested that the dose response curve of danazol was very wide, and 200 mgs daily was likely to be as effective as 400 mgs daily in practice. The capsules were pre-randomised so that there were equal numbers of danazol first and placebo first patients in each block of 6 patients, thus ensuring an equal distribution of both groups on entry. The code-break was kept by the Department of Pharmacy at the University Hospital of Wales in case a life-threatening side

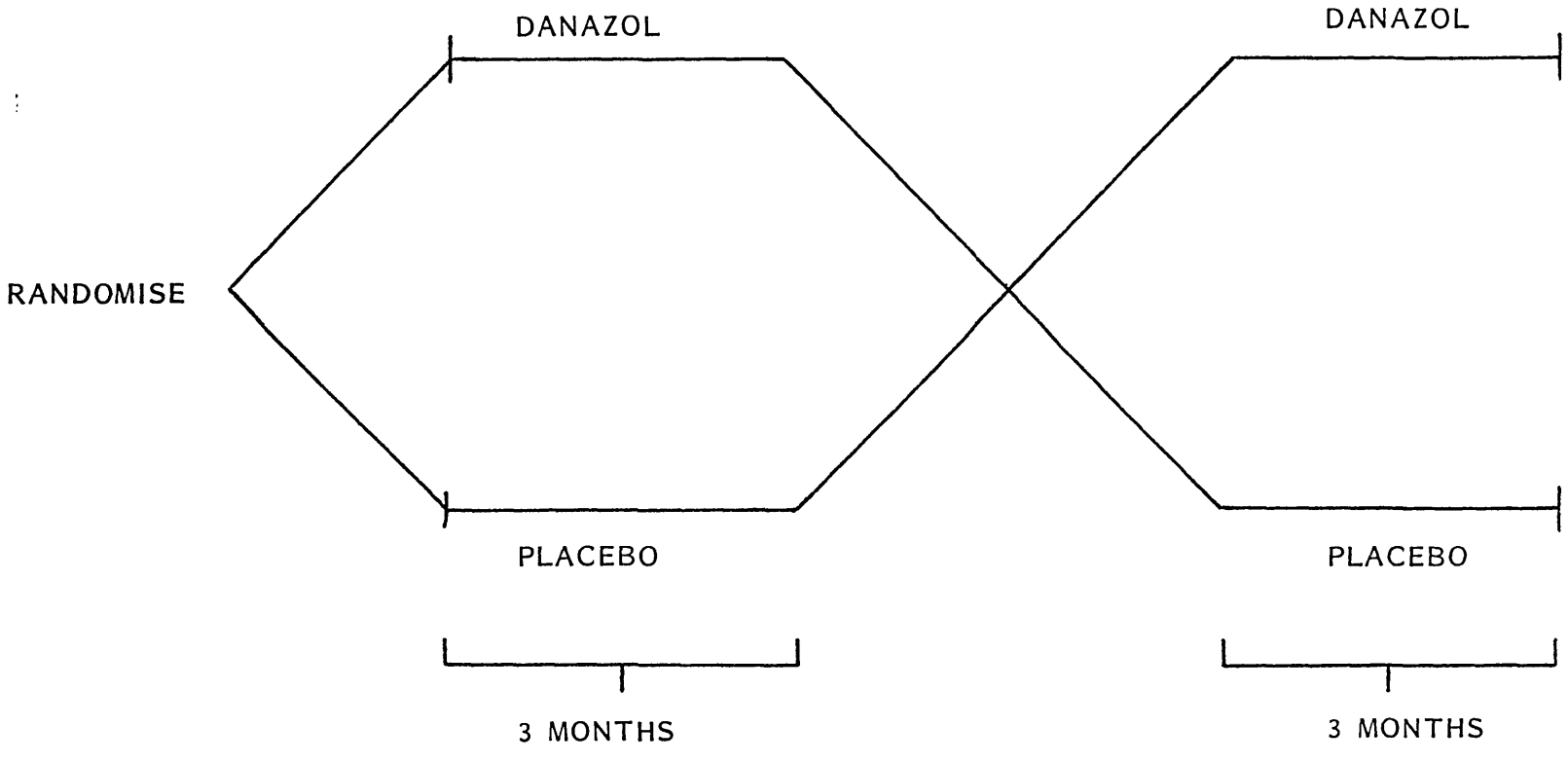


Figure 4.2 THE STRUCTURE OF THE DANAZOL TRIAL

effect required urgent breaking of the code. The code-break was kept in the form of an advent calendar with each patient having a 'window' that could be opened in an emergency without compromising the blindness of the remaining patients. Each bottle contained 60 capsules to allow 30 days treatment. Patients were seen every 28 days, and a new bottle was dispensed at each visit until all 6 bottles had been used. Empty bottles were returned for tablet counts to check compliance. Dosage was designed on a 28 day cycle as it was likely that amenorrhoea would occur in danazol-treated patients; thus the treatment cycle was defined in terms of Bottle 1, Bottle 2, etc., as illustrated on the danazol pain chart shown in fig. 4.3.

Assessment of Response

This was made by pain charts, VLA forms and a clinician's assessment form. Each of these documents was specifically designed for the danazol trial. The pain chart (fig 4.3) was designed to accommodate the trial structure by assessing days of pain in blocks of 28 days, which fitted in with the 30 day treatment course packed in each bottle, allowing a 2 day leeway and with the regular 4 weekly appointments given to each patient. Thus each line of the pain chart was described as Bottle 1, Bottle 2 etc. to make it more comprehensible, as the tablets were issued in bottles labelled simply with the trial and bottle numbers . The pain charts were printed on stiff white card to enhance their durability during the 6 month trial. The pain

BREAST PAIN - DANAZOL TRIAL NO.

Shade in each box on the day you get any breast pain.

For example :-

If you get breast pain on the fifth day of Bottle No. 1 then shade in the square under No. 5 on Bottle 1 line.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Bottle 1																												
Bottle 2																												
Bottle 3																												
Bottle 4																												
Bottle 5																												
Bottle 6																												

Please bring this chart with you on each visit together with your thermometers and the bottle of tablets.

Figure 4.3 THE DAILY BREAST PAIN CHART IN THE DANAZOL TRIAL

charts were then handed in at the end of the trial. The patients were asked simply to shade in any day that they had breast pain.

On entry and at each monthly visit the clinician completed a proforma (fig 4.4) which detailed the assessment visit number, tenderness and nodularity assessed on a simple scale from 1 = no tenderness, 2 = some tenderness, to 3 = marked tenderness, and with response to treatment graded from 1 = asymptomatic, 2 = some improvement, 3 = no improvement, to 4 = worse.

Side effects and tablet count were also ascertained at each visit. The regularity of the menstrual cycle and the patient's weight were recorded since danazol is know to cause irregular menstruation or even amenorrhoea, and can be anabolic. It was clear that enquiry by the assessing clinician of these 2 features could hazard his 'blindness'. In order to reduce this problem, the clinician assessed the patient for tenderness, nodularity and response to treatment only, and the patient's weight, side effects and menstrual cycle were recorded by the clinic nurse after the patient had seen the clinician. These results along with the VLA forms were then filed away immediately in a special trial envelope until data analysis after completion of the trial.

Before trial entry and at each monthly visit each patient completed a one page VLA form (fig. 4.5) which recorded the breast symptoms of pain, discomfort, heaviness, tenderness,

HOSPITAL NO .....

DANAZOL TRIAL

DATE: ..... NAME.....

ASSESSMENT WEIGHT

1,2,3, etc.

Clinical examination

a) Tenderness ☐

0 = Not examined
1 = No tenderness
2 = Some tenderness
3 = Marked tenderness

b) Nodularity or Lumpiness ☐

0 = Not examined
1 = No nodularity
2 = Some nodularity
3 = Marked nodularity

Response to treatment ☐

0 = Not seen
1 = Asymptomatic
2 = Some improvement but residual pain or tenderness present
3 = No improvement
4 = Worse

Side effects:

Tablet count correct/incorrect
If incorrect what discrepancy

Notes - e.g. blood tests taken etc.

Menstrual Cycle
Regular/Irregular/None

Figure 4.4 THE CLINICAL ASSESSMENT FORM - DANAZOL TRIAL

DATE..... DANAZOL TRIAL NAME.....

HOSPITAL.....

VLA FORM FOR ASSESSMENT OF THERAPY IN BREAST PAIN

ASSESSMENT NO..... PATIENT TRIAL NO.....

1, 2, 3, etc.,

PARAMETERS

PAIN

NONE ——— UNBEARABLE

DISCOMFORT

COMFORTABLE ——— UNCOMFORTABLE

FEELING OF WELL BEING

VERY GOOD ——— VERY BAD

IRRITABILITY

NOT AT ALL IRRITABLE ——— EXTREMELY IRRITABLE

BREAST HEAVINESS

NORMAL ——— VERY HEAVY

IS TREATMENT HELPING

NOT AT ALL ——— VERY MUCH

TENDERNESS TO TOUCH

NONE ——— VERY TENDER

DEPRESSION

NO DEPRESSION ——— VERY DEPRESSED

SORENESS

NOT AT ALL ——— VERY SORE

LOSS OF SLEEP

NONE ——— FREQUENT LOSS

Figure 4.5 THE DANAZOL TRIAL VLA FORM

soreness and the general symptoms of well being, irritability, depression and loss of sleep. This VLA form was based on the previous bromocriptine form but had been simplified by inclusion of major breast and general symptoms only. The patient was also asked to assess whether treatment had helped her. The VLA form incorporated a design change from that used in the previous bromocriptine trial as the breast symptoms were interspersed among the general symptoms, unlike the layout of the previous VLA form where all the breast symptoms were grouped together. This was designed to ensure that the patients read the form correctly, as the literature on VLA forms had suggested that patients would tend to mark adjacent lines with similar scores. This tendency could be evaluated on the new form, as 'breast heaviness' was placed adjacent to 'is treatment helping', and it would be clear if patients were tending to group marks, as logically a high score on 'breast heaviness' (i.e. non-improvement) should have a correspondingly low score on 'is treatment helping'. The VLA forms were completed at each monthly clinic visit which were timed to take place in the late luteal phase of each patient's cycle. Occasionally, if a patient was unable to attend on a particular date, a VLA form was mailed to her to be returned by post.

Patients were seen each month in the presumed late luteal phase of the cycle (day 20-28) and the clinician's assessment form and VLA form were completed. Each patient was weighed and details of side effects and menstruation recorded by the clinic nurse. In addition, a venous blood sample was taken

for hormone assay and stored at -20 C after separation of the serum for later batch assay. These blood samples were taken between 9 a.m. and noon and assays were performed at the end of the trial for serum oestradiol, progesterone and prolactin using standard radio-immunoassay techniques.

Statistical Assessment

As in the bromocriptine trial, the clinician's scores were analysed within patient using the Wilcoxon's matched pairs signed ranks test. The VLA scores were analysed by Wilcoxon's signed ranks test as detailed in Appendix I.

The convention was assigned in these analyses that a positive score was given if the danazol score was lower (i.e. symptomatically better) than the placebo score. This was done by subtracting the danazol score from the placebo or pretrial score so that a high pretrial score, say 80, was subtracted from a danazol score, say 30; this would give a score of +50.

The pain chart scores and the hormone levels were grouped and means compared by Student's t test.

4.3 RESULTS

Twenty-eight patients were entered into the study. They had a mean age of 30 yrs (range 20-39), mean weight of 57 Kgs (range 42-88), and the mean duration of breast pain prior to trial entry was 4.1 years (range 1-13). The mean scores $\pm$SD for breast tenderness as rated by the clinican was 2.3$\pm$0.6 and nodularity was 2.4$\pm$0.5, thus confirming the selection criteria of marked tenderness and nodularity. The mean pretrial VLA scores i.e. the patient's rating, confirmed the high level of symptoms with mean scores of 60-80 for most of the symptoms. The randomisation code allocated 12 patients to danazol as the first treatment and 16 patients to placebo first treatment. The age, weight, pain duration and cyle length were not significantly different in the 2 groups, thus confirming random allocation (Table 4.1).

Of the 28 patients who commenced the trial, 6 did not complete the whole course of therapy. The reasons for dropout and the therapy at the time are shown in Table 4.2. It is interesting to note that dropout occurred in every case either on bottle 1 or bottle 2, and no patient dropped out at a later time. Three patients were taking danazol at the time of dropout and 3 were taking placebo. Patients Nos. 5,9, and 20 did not return to the clinic despite several appointments and recall letters; 2 were taking danazol and 1 placebo at the time. Patient No. 22 withdrew voluntarily as she suspected she might be pregnant and on breaking

Table 4.1 Danazol trial patients - entry characteristics

	Means ± SD	
	Danazol -> Placebo N=12	Placebo -> Danazol N=16
Age (years)	28.4±4.7	31.6±5.6
Weight (kgs)	60.2±11.9	55.3±7.7
Pain Duration (yrs)	4.8±3.9	4.1±3.6
Cycle Length (days)	26.3±3.2	27.1±2.2

Table 4.2 Danazol trial dropouts and withdrawals

Trial No.	Bottle No.	Therapy	Reason
5	2	Danazol	Failure to attend
9	2	Danazol	Failure to attend
13	1	Placebo	Severe headaches
20	2	Placebo	Failure to attend
22	1	Danazol	Withdrawn ? pregnant
28	2	Placebo	Calf muscle cramps & finger swelling

the code she was found to be on danazol. The other 2 patients dropped out (Nos 13 and 28) because of severe side effects consisting of finger swelling and calf cramps in patient No. 28, and headaches in patient No. 13. Both of these patients were in fact taking placebo when they dropped out.

When the tablet counts were assessed at the end of the trial it became clear that one patient, No. 23, had not been taking her tablets as her counts showed very high returns with only 12 tablets taken in month 2 and 17 in month 3 out of a projected 60 tablets per month. She was therefore dropped from the efficacy analysis - thus leaving a total of 21 patients who had completed the full 6 months course of the trial. For the reasons discussed in Chapter 2, the efficacy comparisons were made on paired data from the patients who completed the whole 6 months treatment course, but all patients were included in the analysis of side effects.

(a) VLA Results.

Of the 21 patients who completed the trial, 8 were allocated danazol as the first treatment and 13 were allocated placebo as the first treatment. The pretrial VLA scores were compared with those at the end of the placebo period and the end of the danazol period, for each patient for each of the symptoms recorded on the VLA forms. The mean levels for the breast symptoms are shown in figs. 4.6 to 4.9 for both groups

(Fig 4.6 Pain, Fig 4.7 discomfort, Fig 4.8 heaviness, Fig 4.9 tenderness). The raw data are given in Appendix IV. These graphs are simple illustrative as the statistical analysis was performed using the non-parametric Wilcoxon's signed ranks test (Siegel 1956). The results of these analyses are presented as the rank sums for each breast and general symptom recorded on the VLA form (Tables 4.3 to 4.5). In tables 4.3 to 4.5, the signs were allocated so that a higher positive rank sum implied improvement on danazol. Table 4.3 shows that the danazol scores for breast tenderness, heaviness, discomfort and soreness at the end of the 3rd month were all significantly improved compared with pretrial scores in the danazol first group. When the scores at the end of the placebo therapy period were compared with those at the end of the danazol therapy there were no significant changes in any breast symptom. This is not surprising as the scores at the end of the 3 months danazol therapy were very low as shown in figs 4.6 and 4.7 and thus placebo could not really produce any further improvement. In the placebo first group (Table 4.4), the comparison of the pretrial VLA breast scores with the end of the placebo period scores showed a significant improvement in discomfort, heaviness, tenderness, and soreness while on the placebo, again underlining the placebo effect. However, when the danazol scores after the crossover were compared with the end of the placebo treatment scores, danazol was significantly better. The post danazol scores for breast discomfort, tenderness, pain and heaviness, were all significantly better than placebo even though the danazol treatment followed a course of 3 months

placebo therapy which would make it more difficult for danazol to show an effect. In fact, for pain, discomfort and heaviness, the scores on danazol were lower in every patient compared with placebo, resulting in zero negative rank sums on the Wilcoxon's test, thus showing the changes to be in one direcion only, which gives added weight to the statistical interpretation. Thus danazol was significantly better than placebo irrespective of the treatment order, which is an important finding as many studies of chronic pain using the crossover technique have shown that the first treatment given, often has an advantage over the 2nd treatment, so that if there is little to choose between the 2 therapies the first treatment would apparently fare better. However, these results demonstrate that danazol was superior to placebo even when it followed placebo therapy.

The effectiveness of danazol was also demonstrated when a treatment interaction analysis was made using the Mann-Whitney-Wilcoxon test. This gave a significant difference for pain (X2=4.7), suggesting a definite carry-over effect of danazol (also seen in fig. 4.7). Comparison of the 400mgs with the 200mgs dose of danazol (fig. 4.11), showed similar improvement on both doses. The differences between the 400mgs and 200mgs doses were not statistically different at each time point using an unpaired Wilcoxon test for independent samples.

Analysis of the general symptoms on the VLA form showed

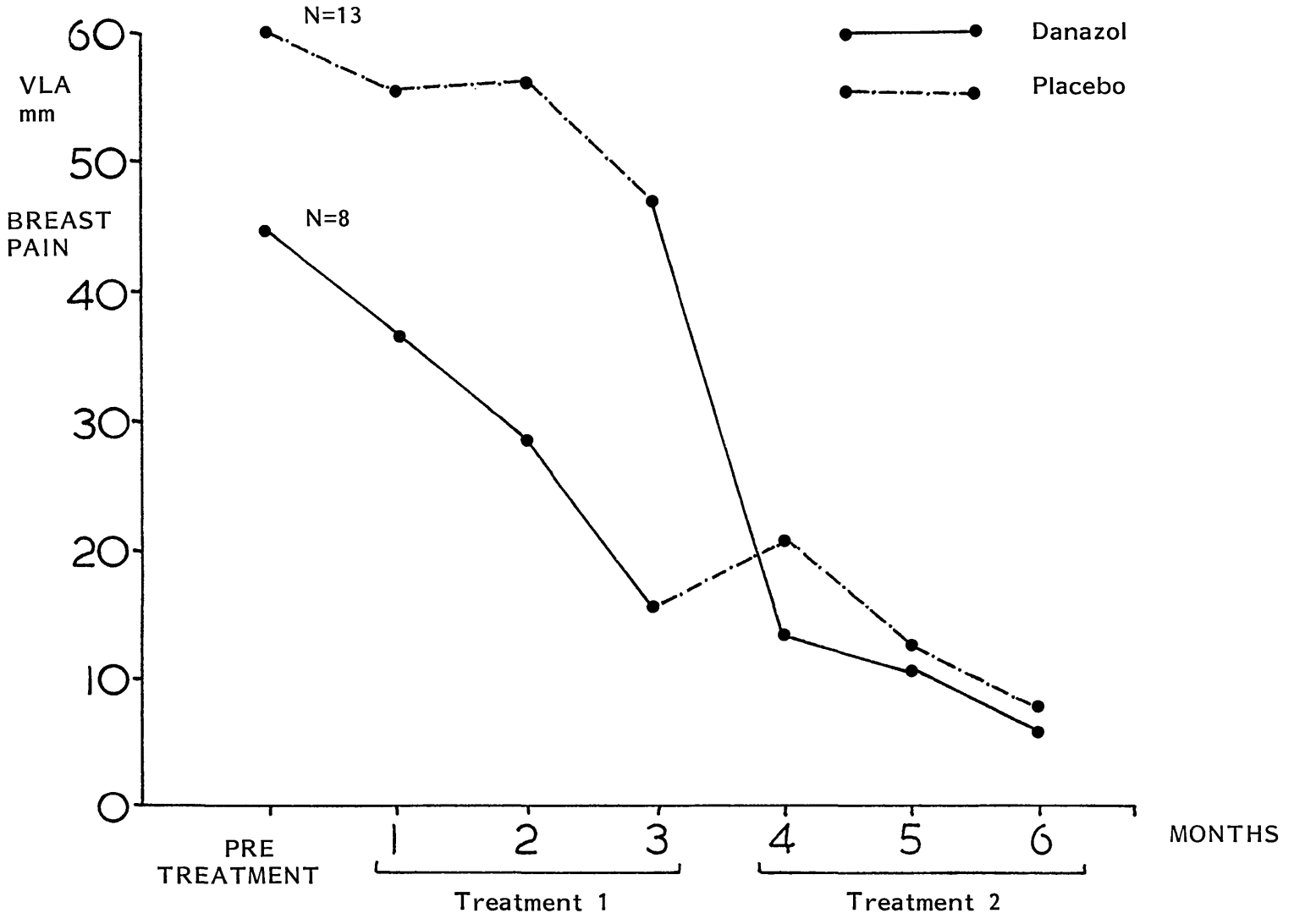


Figure 4.6 The mean VLA scores for breast pain in the 2 groups in the danazol trial. The crossover occurred at the end of the 3rd month.

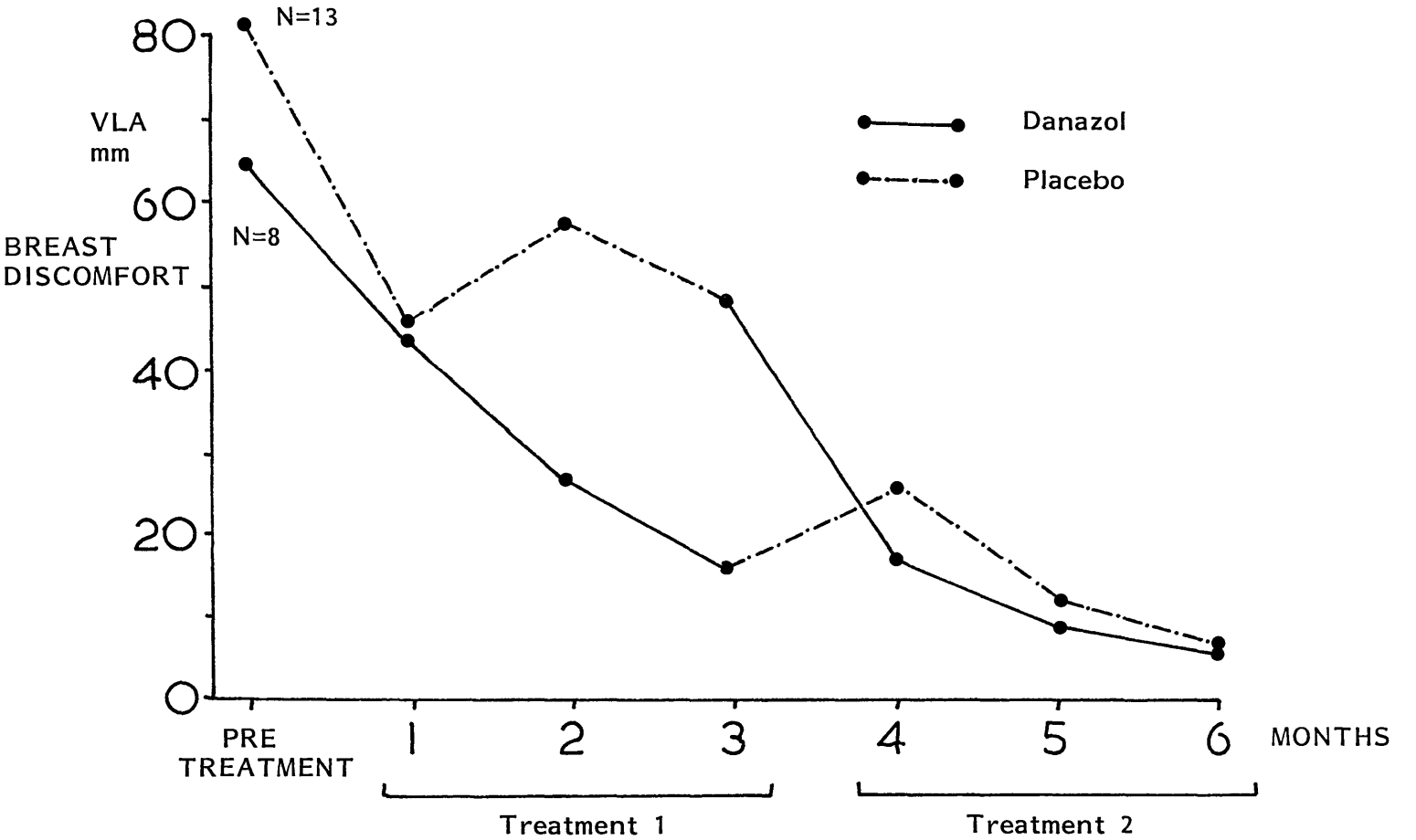


Figure 4.7 The mean VLA scores for breast discomfort in the 2 groups in the danazol trial. The crossover occurred at the end of the 3rd month.

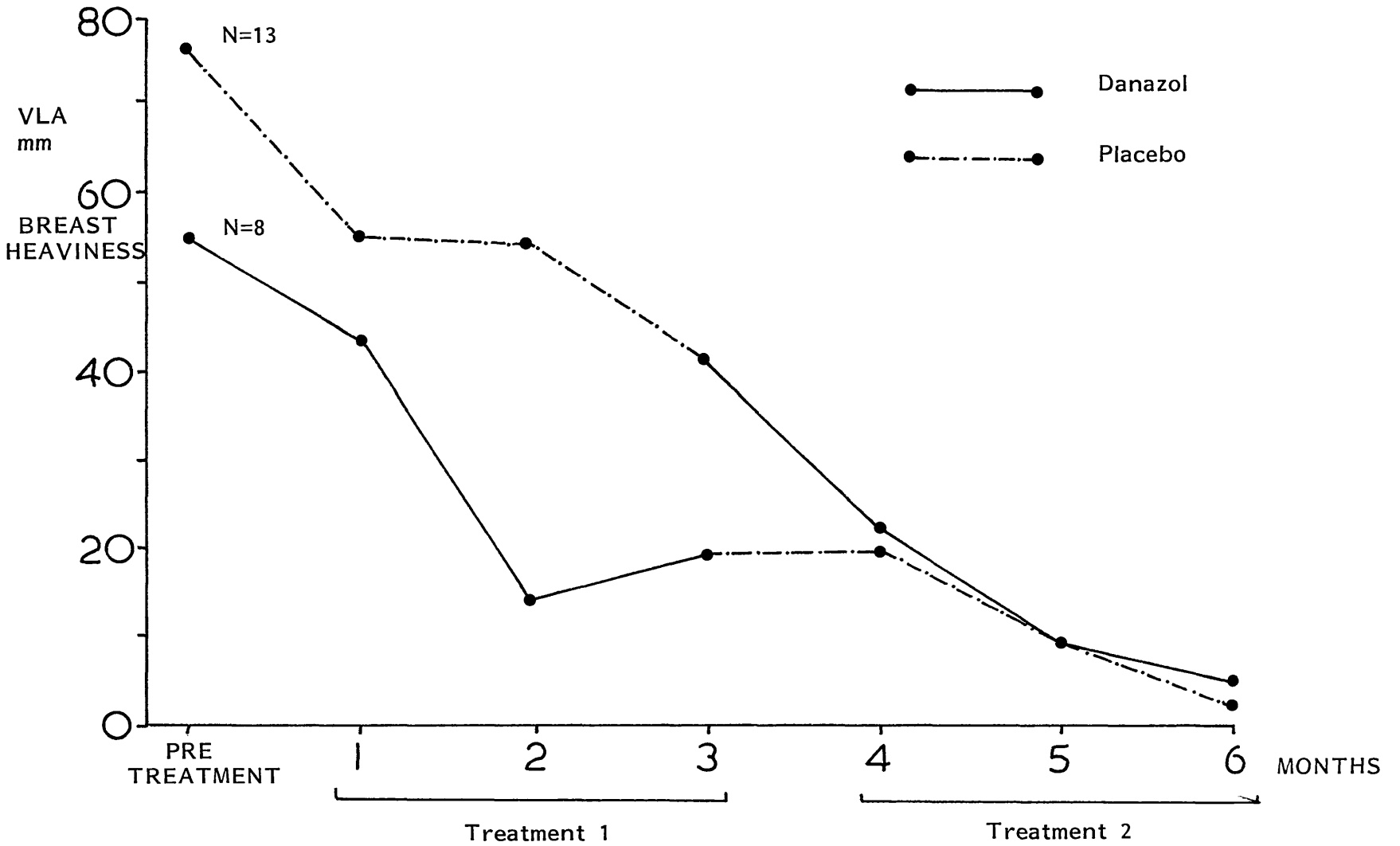


Figure 4.8 The mean VLA scores for breast heaviness in the 2 groups in the danazol trial. The crossover occurred at the end of the 3rd month.

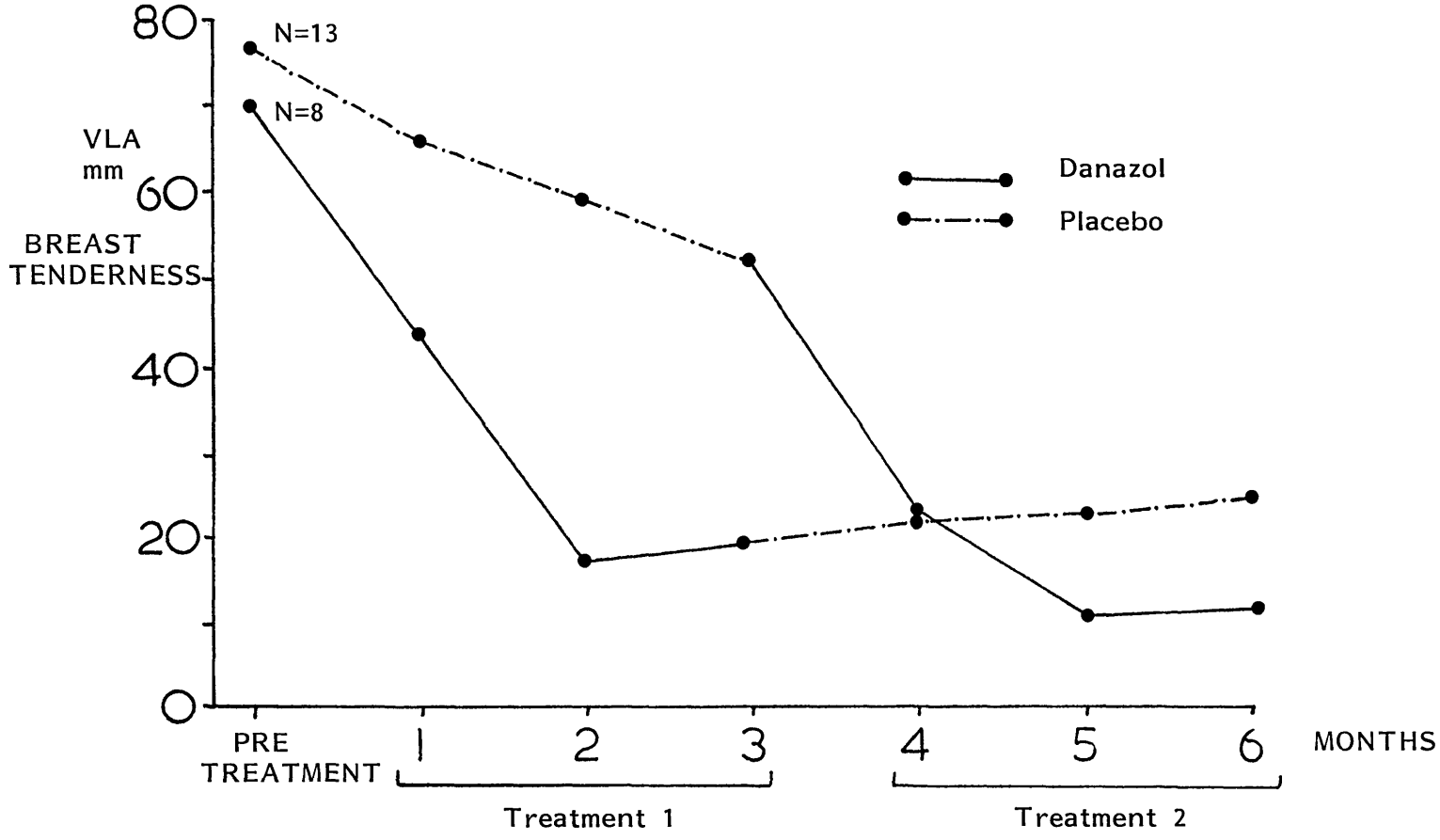


Figure 4.9 The mean VLA scores for breast tenderness in the 2 groups in the danazol trial. The crossover occurred at the end of the 3rd month.

Table 4.3 VLA Analyses Wilcoxon's Rank Sums - Danazol -> Placebo Group

	Rank Sums N=8	
	Pre Trial v Danazol	End Danazol v End Placebo
Breast pain	-4 +32	-3 +7
Breast discomfort	-0 +36*	-3 +7
Breast heaviness	-1 +35*	-1 +9
Tenderness to touch	-0 +36†	-10 +5
Breast soreness	-1 +20†	-6 +4

* $p < 0.01$)
† $p < 0.05$) Wilcoxon's matched pairs signed ranks test

Table 4.4 VLA Analyses Wilcoxon's Rank Sums - Placebo -> Danazol Group

	Rank Sums N=13					
	Pre Trial v End Placebo			End Placebo v End Danazol		
Breast Pain	-31.5	+59.5		- 0	+91†	
Breast Discomfort	-11	+80	*	- 0	+78†	
Breast Heaviness	-12	+77	*	- 0	+66†	
Tenderness to touch	-16.5	+74.5	*	-12	+77	*
Breast Soreness	-13	+78	*	- 2	+76†	

* $p < 0.05$ Wilcoxon's signed rank test for paired observations

† $p < 0.01$ Wilcoxon's signed rank test for paired observations

significant improvement in the placebo first group of well being and irritability both on placebo and then on subsequent danazol (Table 4.5). This is not surprising as significant improvement of breast pain should be paralleled by an improved feeling of well being and less irritability. The patients also recorded significant improvement in favour of danazol on the rating of depression (Table 4.5).

(b) Clinician's rating - simple scale.

This was analysed using the Wilcoxon's matched pairs signed ranks test and results are shown in both groups in tables 4.6 and 4.7. In the danazol first group (table 4.6), there was a significant fall in tenderness from 2.37±0.74 (mean ± SD) to 1.37±0.74 ($p<0.01$ Wilcoxon's signed ranks test). Also nodularity was significantly improved by 3 months danazol therapy from a pretrial value of 2.5±0.53 to 1.62±0.51 ($p<0.01$ Wilcoxon's signed ranks test). The improvement on danazol was progressive with the lowest scores being obtained at the end of 3 months therapy. The response to therapy score was also better on danazol although this did not reach statistical significance. In the placebo first group (Table 4.7) the pretrial mean±SD scores for tenderness (2.23± 0.59) and nodularity (2.15±0.55) were not significantly improved by a 3 month course of placebo therapy: tenderness (2.00±0.67) and nodularity (1.76±0.72). However after the crossover and 3 months therapy with danazol, both the tenderness (1.07±0.27) and nodularity (1.16±0.38) scores were

Table 4.5 VLA Analyses - General Symptoms - Both Groups

Danazol -> Placebo Group

N=8

	Rank Sums	
	Pre Trial v end danazol	End danazol v end placebo
Feeling of well being	-11 +25	- 6 +22
Irritability	- 6 +22	- 7 +21
Depression	- 9 +12	-13 +15
Loss of sleep	- 6 + 9	- 5 + 5

Placebo -> Danazol group

N=13

	Rank Sums	
	Pre trial v end placebo	End placebo v end danazol
Feeling of well being	-10 +81*	-12 +66*
Irritability	- 9 +82*	-12 +79*
Depression	-54 +37	-15 +63*
Loss of sleep	-19 +59	-24 +42

* $p < 0.05$ Wilcoxon's signed rank test for paired observations

Table 4.6 CLINICIAN'S RESULTS - DANAZOL → PLACEBO GROUP

	CLINICIAN'S SCORES (MEAN ± SD)		
	TENDERNESS	NODULARITY	RESPONSE TO THERAPY
PRE-TRIAL	2.37 ± 0.74	2.50 ± 0.53	N/A
END OF DANAZOL	1.37 ± 0.74*	1.62 ± 0.51*	1.63 ± 0.92
END OF PLACEBO	1.50 ± 0.53	1.71 ± 0.48	2.0 ± 0.78

* Danazol v pre trial score $p < 0.01$ (Wilcoxon's matched pairs signed ranks test)

Table 4.7 CLINICIAN'S RESULTS - PLACEBO → DANAZOL GROUP

	CLINICIAN'S SCORES (MEAN ± SD)		
	TENDERNESS	NODULARITY	RESPONSE TO THERAPY
PRE TRIAL	2.23 ± 0.59	2.15 ± 0.55	N/A
END OF PLACEBO	2.00 ± 0.67	1.76 ± 0.72	2.46 ± 0.78
END OF DANAZOL	1.07 ± 0.27*	1.16 ± 0.38*	1.23 ± 0.44

* End of danazol v end of placebo $p<0.05$ (Wilcoxon's matched pairs signed ranks test)

significantly improved ($p < 0.05$ Wilcoxon's matched pairs signed ranks test). The score for response to therapy at the end of the danazol period (1.23 ± 0.44) was also significantly lower compared with the end of placebo period (2.46 ± 0.78 $p < 0.05$ Wilcoxon's matched pairs signed ranks test). Thus, as in the VLA data for breast symptoms, danazol significantly improved breast symptoms irrespective of treatment order. There was a small placebo effect in this group as the placebo scores were lower compared with the pretrial scores, although the improvement did not reach statistical significance.

(c) Pain Charts.

The data from the pain charts was recorded as days of pain for each 28 day cycle of treatment (Fig 4.10). Only 13 complete pain charts were returned by the patients; these are analysed. In the danazol first group there was a reduction from a pretrial mean score of 13.2 days pain to a mean of 2.5 days at the end of 3 months treatment. When the crossover to placebo occurred there was an immediate increase to a mean of 7 days of pain which then remained static. Analysis of these data by Student's 't' test showed a significant improvement in favour of danazol. In the placebo first group, there was very little evidence of a placebo effect as the mean days of pain fluctuated around 12 days after starting at a pretrial mean of 13.5 days. However, after the crossover there was a dramatic fall in mean

DANAZOL TRIAL - PAIN CHART RESULTS

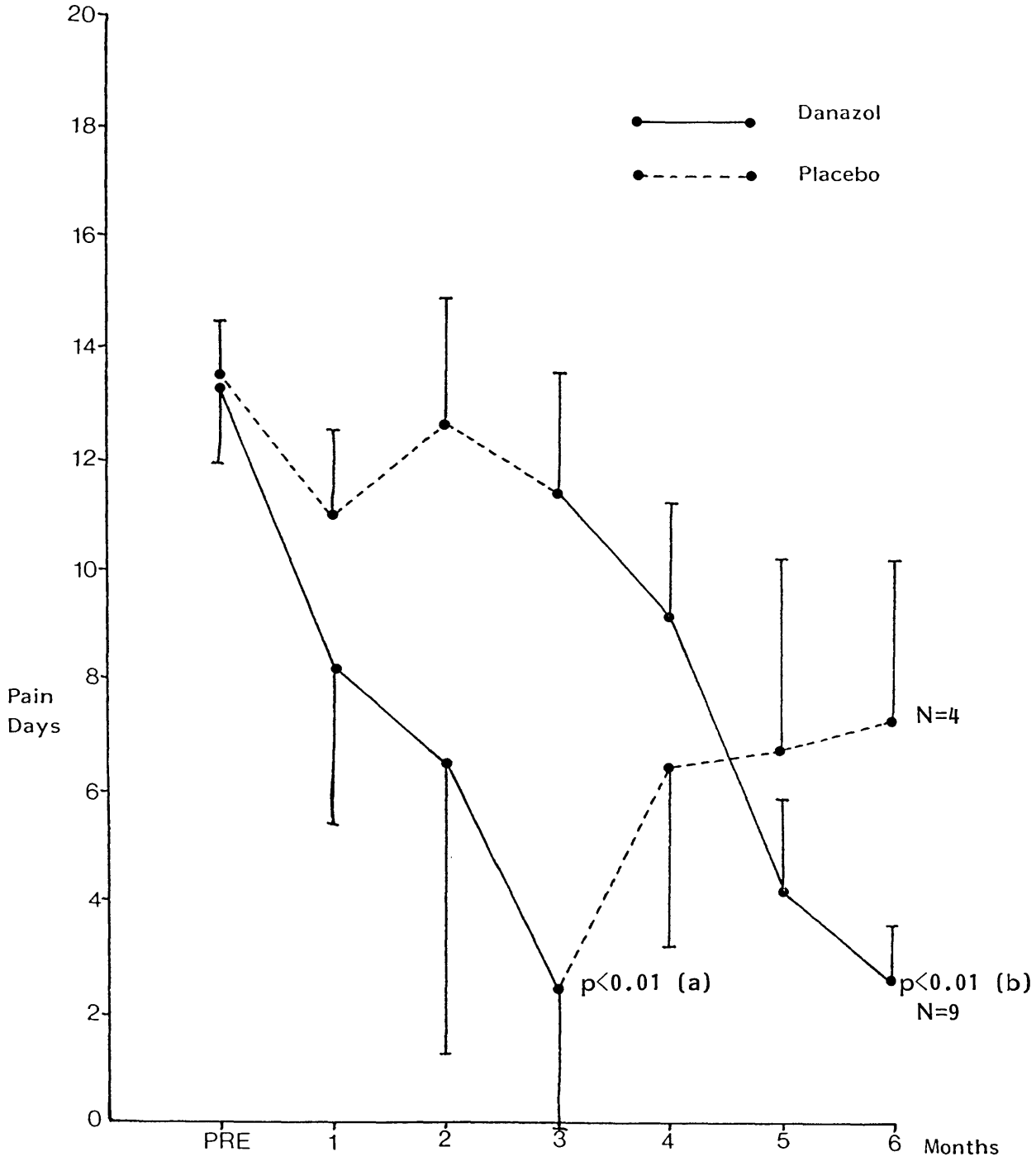


Figure 4.10 The pain chart results in the danazol trial shown as days of severe pain/month. The error bars indicate 1 standard error. The p values refer to comparison between danazol and pretrial values (a) and placebo versus danazol (b) using Student's t test.

days of pain from 11 to 3 after 3 months danazol therapy, a highly significant change. The slopes of the danazol curves run parallel showing a similar rate of improvement in the danazol treatment group irrespective of the treatment order, as shown in the VLA analyses also.

(d) Side Effects.

There were 3 separate assessments of side effects as detailed in the methods section. The clinician's form listed all the side effects except weight gain and menstrual disturbance as these were recorded by the nurse. Overall patients experienced side effects in 30% of the months on placebo treatment and 38% of the months on danazol treatment, but there was a marked treatment order effect as the first treatment resulted in a higher reporting rate than the second treatment (Table 4.8). Twenty of the 21 months where side effects were reported on placebo therapy, occurred in the first 3 months of placebo treatment and 17 of the total 26 danazol side effect months occurred when danazol was the first therapy. The common symptoms of headache, tiredness, depression, dizziness and nausea were reported in both the danazol and placebo patients, and it is clear that it would have been difficult for the clinician to tell which treatment was first and thus the blindness of the study was probably preserved. Although the total side effects were higher on danazol (38% of all treated months v 30% for placebo), the number of dropouts was identical on both treatments (Table 4.2), suggesting that there was little

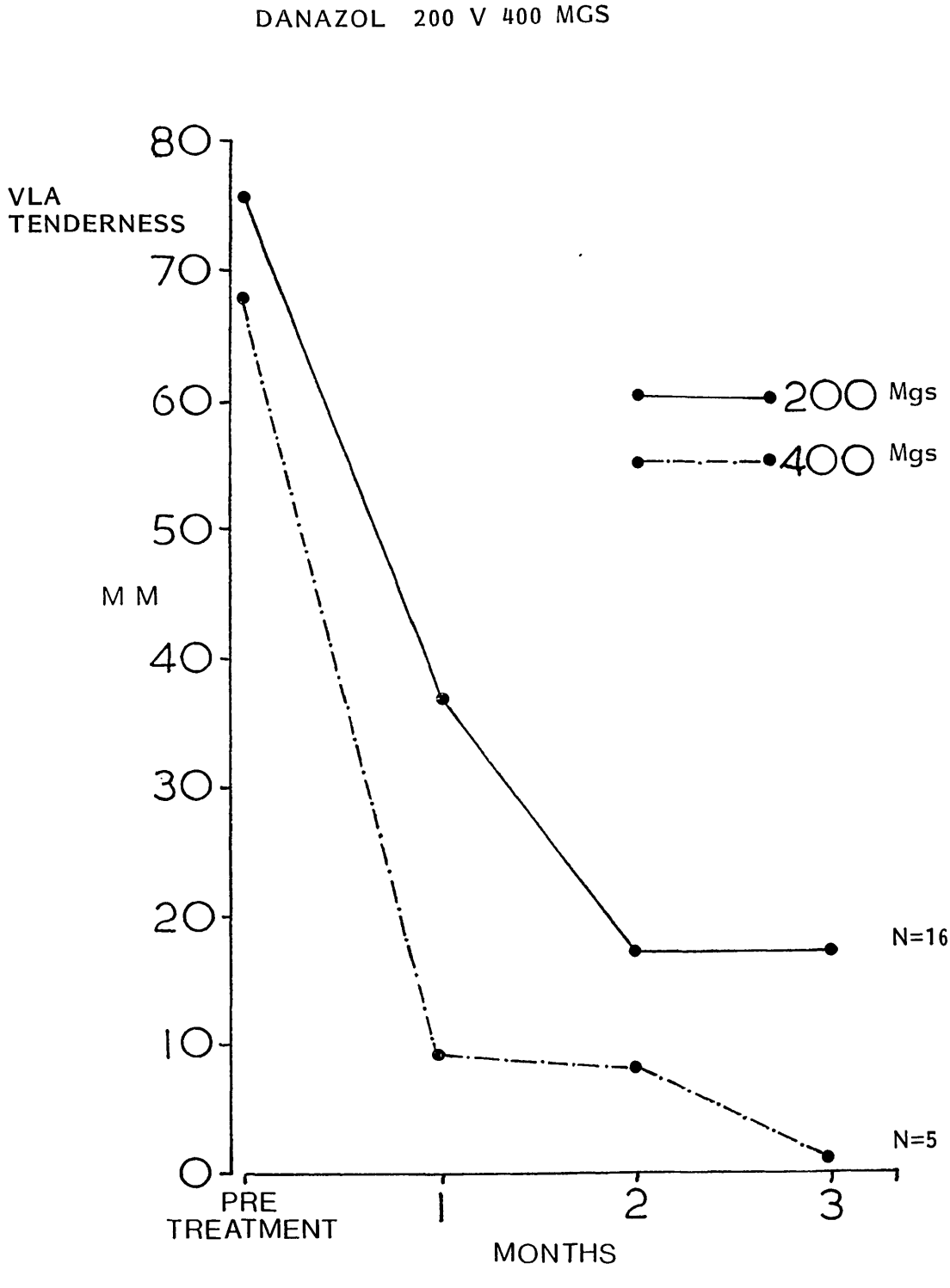


Figure 4.11 The mean VLA scores irrespective of treatment order for breast tenderness in 5 patients on 400 mgs/day compared with 16 patients on 200 mgs/day.

Table 4.8 Danazol trial - Non menstrual side effects

	Placebo months Total No = 71	Danazol months Total No = 69
Total side effect months	21 (30%)	26 (38%)
No of side effect months on 1st therapy	20	17
Individual side effects		
Headache *	11	12
Depression	4	1
Nausea *	2	7
Dizziness *	2	1
Tiredness	2	1
Oily skin	-	1
Weight gain	-	2
Calf cramps *	1	1

* Symptoms causing withdrawal from the trial

practical difference between the 2 therapies. All the dropouts occurred in the first 3 months of therapy again suggesting a marked treatment order effect. Only the symptoms of oily skin and weight gain occurred exclusively in the danazol months, but were only reported by 3 patients.

Turning to the anticipated side effects of danazol, there were obvious differences in the incidence of weight gain (anabolic effect) and disturbance of menstruation (anti-gonadotrophic effect) between danazol and placebo. Of the 21 patients who completed the full course of danazol, 6 noted menstrual irregularity and 10 had complete amenorrhoea on danazol (Table 4.9). The changes in menstrual pattern on placebo were limited to mild irregularities in 2 patients and amenorrhoea in one, when the placebo treatment had followed danazol therapy. Thus, as expected, menstrual changes were significantly increased on danazol therapy ($p < 0.05$ Chi square test).

The 21 patients who completed the trial were weighed monthly for the duration of the trial and the raw data are shown in Appendix IV. Analysis of the weight changes during the trial (Table 4.10) showed no significant changes in the weights on placebo compared with the pretrial values, whereas the majority of patients on danazol gained weight, although the mean gain was only 1.7 Kgs compared with placebo (Table 4.10). Only 3 patients lost weight while on danazol, thus demonstrating the mild anabolic effect of the drug in the majority of the patients.

Table 4.9 Danazol Trial - Menstrual Effects

	Pre trial	Placebo	Danazol
Menstrual Cycle			
Regular	20	18	5
Irregular	1	2	6
Amenorrhoea	0	1	10*

* $p < 0.05$ X_2 test versus pre trial or placebo

Table 4.10 Weight change during the danazol trial in kilograms

Trial No	Pre Trial	Placebo (mean)	Danazol (mean)	Danazol Change v pre trial
1	58.0	57.1	56.3	-1.7
2	47.5	46.8	48.2	+0.7
3	88.7	86.7	90.8	+2.1
4	60.6	60.3	61.0	+0.4
6	51.0	51.2	54.7	+3.7
7	45.2	45.4	47.0	+1.8
8	60.1	62.2	62.0	+1.9
10	44.5	43.5	44.6	+0.1
11	53.5	53.0	54.8	+1.3
12	50.5	52.0	51.3	+0.8
14	52.2	56.7	59.5	+7.3
15	46.5	45.6	48.5	+2.0
16	58.7	58.8	60.5	+1.8
17	73.1	72.5	71.8	-1.3
18	65.4	65.5	68.8	+3.4
19	53.6	56.5	57.4	+3.8
21	61.0	63.5	66.2	+5.1
24	61.5	62.4	64.7	+3.2
25	42.0	42.0	44.4	+2.4
27	54.6	53.5	53.3	-1.3
29	59.0	59.0	59.0	0
	56.5±10.6	56.8±10.4	58.3±10.7	1.7±2.1

However, as can be seen from the large standard deviations many women varied by 3-4 Kilograms on a month to month basis, and only 2 patients complained of weight gain as a specific side effect.

(e) Hormone assays.

The 2 ovarian steroids progesterone and oestradiol,and the pituitary hormone prolactin, were assayed in 16 patients who had reasonably complete samples available. Very few samples were available on the other 5 patients and only the pretrial samples were available on the dropout cases as they dropped out early in the study.

The prolactin values (mean $\pm$ SD) are shown in Table 4.11, for both groups. These show no significant change on danazol with mean values varying from 120-199 mU/L on danazol and 154-263 mU/L on placebo. There is perhaps a suggestion that prolactin levels are slightly lower on danazol, but the large number of missing values (due to loss in a centrifuge) makes interpretation difficult.

Progesterone levels, however, (Table 4.12) show the profound suppression expected on danazol therapy, with mean levels of around 2-5 ng/ml in the luteal phase. In the placebo first group there was no effect of placebo on progesterone levels with typical luteal levels of 20-30 ng/ml being recorded in months 1-3. When the patients crossed over to danazol, mean levels of

progesterone fell to 2.1-2.8 ng/ml. Thus the expected effect of suppression of ovulation was confirmed in the patients while taking danazol, but the progesterone levels began rising towards the end of the 3 months period in the patients who took placebo after danazol. This showed that the ovarian suppression is temporary and predicts that symptoms will return in many patients as the drug has only produced a temporary change in the hormonal milieu. However, the response was apparently different in some individuals as patients Nos. 3, 8, 12 had normal luteal progesterone levels within 1 month of stopping danazol, while patients No. 12 and 25 took 2 months to return to normal luteal progesterone levels. Patient No. 16 apparently did not return to normal ovulation even after 3 months placebo treatment, although she had had a normal luteal progesterone pretrial. The pretrial levels suggested that 5 patients out of the 12 may have had an anovulatory cycle in the month preceeding the start of the trial, yet they all had severe symptoms as shown on their VLA scores. This would be consistent with the literature and previous experience in the Cardiff Mastalgia Clinic, which shows no evidence of a direct correlation of symptoms with hormone levels; indeed the Mauvais-Jarvis data suggests anovular cycles may equally cause mastalgia (Mauvais-Jarvis et al 1979).

The oestradiol data were very surprising at first sight as the levels of oestradiol were apparently very high in these patients (table 4.13). However, it became clear that danazol cross-reacted with the anti-oestradiol antibody used in the assay

and thus gave very high readings when the patients were taking danazol. Thus the assay was really measuring oestradiol + danazol levels. There was insufficient serum available to repeat the assay using a different system, so that a measure could not be obtained for oestradiol. However this problem is not quite the disaster it may seem for 2 reasons. First, there is no evidence in the literature for any abnormality of oestradiol levels in mastalgia (Wang and Fentiman 1985), secondly, the assay does allow a measure of compliance as serum danazol levels have accidentally been measured.

The assay did show good compliance as the levels of oestradiol/danazol in the placebo first group show an increase from a mean of 384-605 pg/ml in months 1, 2 and 3 to a mean of 1,314 to 1,598 in months 4, 5 and 6, thus confirming that the patients were taking danazol regularly (Table 4.13). All 9 of the patients who had complete samples showed an increase in their serum oestradiol/danazol levels in months 4, 5, 6 showing good compliance. The pretrial levels in the placebo first group showed no effect of placebo on oestradiol levels with a mean of 570 pg/ml pretrial compared with similar means for months 1, 2 and 3. These figures can be taken as indicating true oestradiol levels as there was obviously no danazol in the serum as patients were taking placebo at the time.

In the danazol first group, the compliance was equally good as the mean level of oestradiol/danazol in months 1, 2, 3

were significantly higher than the pretrial mean of 435 pg/ml (438 v 1,398, 1,040 and 1,363 $p < 0.0001$ t test). Again all the patients individually showed elevated oestradiol/danazol levels in months 1, 2, 3 compared with their pretrial figures. It is of interest to see that danazol cleared rapidly from the serum in the patients who took danazol first, as the levels of oestradiol had returned to normal in month 4 and remained at a normal level in months 5 and 6. This would fit with the half life and clearance data on danazol, but is interesting as the return of symptoms is much slower. Thus the serendipity of an assay cross-reaction has given the proof that these patients were taking the drug and that the randomisation packaging was correct.

Table 4.11 - Hormone Results - Prolactin

Mean ± SD in mU/Litre

	Pre Trial	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Placebo -> Danazol N=9	174±84	154±33	213±43	159±53	120±51	152±38	141±54
Danazol -> Placebo N=7	185±47	165±62	199±48	171±51	211±60	253±46	263±50

No significant differences

Table 4.12 - Hormone Results - Progesterone

Mean ± SD in ng/ml

	Pre Trial	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Placebo -> Danazol N=9	18.3±17.4	30.5±13.6	26.9±18.0	22.8±19.0	2.8±3.3*	5.1±6.6*	2.1±2.9*
Danazol -> Placebo N=7	24.1±17.8	1.34±0.5*	2.0±2.4*	1.3±0.4*	8.39±8.7	12.9±11.4	13.7±10.8

* p <0.001 danazol v pretrial Student's t test

Table 4.13 - Hormone Results - Oestradiol

Mean ± SD in picograms/litre

	Pre Trial	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Placebo -> Danazol N=9	570±376	605±315	539±194	384±218	1598±399	1314±516	1566±308
Danazol -> Placebo N=7	438±185	1398±234	1040±468	1363±348	265±199	295±123	347±152

Danazol v. placebo $p < 0.001$ Student's t test
Pretrial v. danazol $p < 0.001$ Student's t test

4.4 DISCUSSION

The trial reported herein has shown that danazol at doses of 200 mgs or 400 mgs daily given over 3 months reduces both breast tenderness/pain and nodularity when compared with placebo therapy, irrespective of the order in which it is given. A definite placebo effect has been demonstrated especially in the patient's assessment of her response to therapy. The variables of placebo effect and assessment methods had not previously been explored carefully, although several open studies have been reported. Peters et al reported a study of 24 patients with severe mastodynia treated with 600 mgs of danazol per day for 6 weeks, and reported complete resolution of pain in 71% (Peters et al 1980). They also noticed a placebo response rate of 21% in a preliminary placebo treatment cycle, but the study was not blind to the clinician. Dhont et al carried out a double blind randomised study in 25 patients and showed that 400 mgs per day of danazol was significantly better than placebo treatment for relieving breast symptoms and nodularity (Dhont et al 1979). The present trial has also shown that a dose of 200 mgs daily is as effective as a dose of 400 mgs, which has a higher incidence of side effects. This efficacy at a lower dose has recently been confirmed by a double blind Swedish/Danish study (Doberl et al 1984) where a dose of 100mgs a day was as effective as 200mgs a day. A recent double blind study from France of 38 patients with mastodynia using an identical trial structure to that in this thesis, also confirmed the efficacy of danazol at 400 mgs/day, but

noted side effects in 75% of the patients (Gorins et al 1984).

Watts et al found improvement in breast pain in a group of patients with premenstrual syndrome who were treated with danazol in a double blind, placebo-controlled study, but 12 out of 40 patients withdrew because of side effects (Watts et al 1985).

As a direct result of the study reported here the dose recommendation for danazol in the indication of benign breast disease, has been revised downwards to 300 mgs daily (Winthrop data sheet 1983). In a recent review London et al also recommended lower doses of 100-200 mgs daily in view of the cost and the continued efficacy of danazol at lower doses (London et al 1982). This trial has shown that the VLA system as modified from the previously longer version used in the bromocriptine trial (Appendix I), worked well and correlated with the pain chart and physician's rating, although the latter technique is less sensitive. VLA systems have the advantage that the assessment can be done by post and the patient need not be seen each month, which also applies to the pain chart which is simple to understand and was completed satisfactorily by all the women in this trial. The trial has shown effective suppression of symptoms which is progressive up to 3 months with very low levels being reached at the end of 3 months therapy. However, it is clear from the group who took placebo as a 2nd therapy that symptoms begin to return

within 3 months in some patients, even though ovulation returns sooner in some. It would appear that danazol has a 'carry-over' effect as the symptoms take longer to return than the ovary takes to recover from the suppression. This may well be an expression of our lack of understanding of the mechanism of action of danazol, as discussed in the introduction.

These results do agree with the published work that danazol causes suppression of the menstrual cycle in humans (Greenblatt et al 1971). The low progesterone levels show that ovulation was suppressed in these patients at a dose of 200 mgs daily and show the wide response curve of danazol, as Greenblatt used a dose of 800 mgs daily in his initial studies (Greenblatt et al 1971).

At 800 mgs daily Greenblatt and co-workers noted that 74% of their patients became amenorrhoeic within 1 month and 95% at 3 months, in contrast to this study at 200-400 mgs daily which showed a much lower incidence of 48% amenorrhoea at 3 months. Peters reported a 100% rate of amenorrhoea on 600 mgs of danazol per day taken for 6 weeks in 24 patients with mastodynia (Peters et al 1980). Thus it appears that danazol at high dosage causes complete amenorrhoea, but although reduction in dosage decreases the incidence of amenorrhoea, the therapeutic effect for breast symptoms at least, does not appear to be lost. It is clear from the progesterone results of this trial, that lower dosages of danazol are still producing suppression of the ovarian cycle even

if menstruation continues. The explanation may be that high dosages of danazol suppress basal levels of pituitary gonadotrophins, while lower levels simply suppress the midcycle surge and hence prevent ovulation but not menstruation. But, this explanation is probably far too simple if all the possible actions of danazol are considered, and there are many other possible explanations. Recent work by Forbes et al shows that danazol profoundly suppresses sex hormone binding globulin (SHBG) levels, and this effect occurs even at very low dosages (Forbes et al 1986). Depression of SHBG levels would theoretically increase the levels of free steroid hormones in the serum thus enhancing their activity, and it is thus possible that in women this would liberate androgens which are normally tightly bound and thus produce a therapeutic effect (and side effects) by increasing androgenic tone. Indeed Nilsson et al were able to demonstrate an increase in free but not total testosterone on danazol treatment (Nilsson et al 1983). Since Forbes et al have shown that suppression of the SHBG levels continues for several months after danazol has been stopped, presumably due to slow hepatic synthesis, this might explain the prolonged carry-over effect of the drug (Forbes et al 1986). It seems likely that a combination of mechanisms result in the therapeutic effect of danazol.

This trial has also shown that breast nodularity was reduced, albeit at a slower rate than pain. This observation would fit in with the observations of patients who noted a decrease in breast size (Lauerson et al 1975, Peters et al 1980).

The slower response of nodularity is perhaps not surprising, as breast nodularity is due to a variety of histological changes with fibrosis, adenosis and cystic change predominating. The precise changes that danazol induces in each of these histological components is unknown, but it is clear that reversal of dense fibrotic change would be a more prolonged process than reduction in oedema. Since it has been shown that mastalgia is not associated with any particular histological picture (Watt-Boolsen et al 1982),it would be difficult to predict a specific change in any particular component as a result of danazol therapy. The reactivity of the target tissue (breast) to individual drugs is ill-understood, and a study of the oestrogen reactivity of benign breast tissue in mastalgia patients using monoclonal antibody immunohistochemistry is detailed in the next chapter of this thesis. Recent work has shown that ultrasonographic density in the breast is unrelated to symptoms, as the same changes occur in painful and non-painful breasts equally (Ayers and Gidwani 1983). This fits in with clinical experience as it is known that lumpy breasts commonly occur without mastalgia and vice versa. It appears that breast pain is more related to some hormonal abnormality rather than to a structural problem, as it has been shown that patients with cyclical mastalgia have an abnormality of stimulated prolactin levels (Kumar et al 1984a+b, Watt-Boolsen et al 1985). It is not clear whether patients with nodularity alone would benefit equally from danazol therapy, although this would appear to be the case from clinical experience with the drug. It has been claimed recently that both mammographic density (Kissin

et al 1984, Tobiassen et al 1984) and cyst formation are reduced by danazol (Hinton et al 1986b). This would suggest that danazol reduces several components of the histological picture of benign breast disease, and as a result some have claimed that the drug can be used to 'clear' densities in the breast in order to improve the differential diagnosis of small breast cancers in nodular breasts (Greenblatt et al 1982). Whether this concept is tenable is not clear, and further work in this area is required.

The controlled trial showed an overall side effect incidence, excluding menstrual change, of 30%. It is debatable if altered menstruation should be called a side effect, since it is the expected therapeutic action of the drug and can be predicted with reasonable accuracy. The incidence of side effects on a month by month basis was slightly higher on danazol (38 v 30%) but only nausea was commoner. The overall incidence of 30% is consistent with many other studies and a range of 18-60% was reported in a recent review (Mansel 1987). The common side effects are: headache, acne, muscle cramps, weight gain and reduction in breast size. In a review of 704 patients mostly at a dosage of 800 mgs daily, the incidence of breast change, acne, headache and muscle cramps was 4%, 13%, 7% and 4% respectively (Spooner 1977). Many of these side effects are minor and are readily tolerated by the patients, and a more meaningful figure is the dropout rate where patients are unable to continue taking the drug. The dropout rate in published studies has varied from 6-20% with an average figure of 9% (Mansel 1987).The trial reported here

has one of the highest dropout rates at 20%, but it should be remembered that only half of the dropouts occurred on danazol, and so the true dropout rate is around the average published figure of 10%. Tobiassen et al reported dropout rates of 5% at 200mgs and 18% at 400mgs in an open study of 124 patients with fibrocystic disease (Tobiassen et al 1984). This study does show the importance of placebo control as 3 patients developed symptoms severe enough to make them discontinue therapy, yet they were only taking placebo at the time. In another study Doberl et al found a higher incidence of side effects on placebo than on danazol (Doberl et al 1984).

Weight gain is often mentioned by the patients on danazol and the data confirm there is generalised weight gain on the drug as all but 3 women put on some weight (Table 4.10). However, the increase is relatively small (around 2 Kgs) as in a recent study (Tobiassen et al 1984) and even at 800 mgs the weight gain is limited to 2 Kgs, and tends to decrease after 4 months therapy. (Lauerson et al 1975). The limitation in weight at entry to the danazol trial may have contributed to the small overall increase seen in these patients. Powles et al recorded weight gains ranging from 1.5 to 6 Kgs at a dose of 200 mgs daily and reported that 72% of their patients noted weight gain (Powles et al 1987), figures which are identical to the Cardiff study.

Amenorrhoea occurs predictably in about 100% of women at 800 mgs, but as this study shows, this reduces to about 30% at 200

mgs daily. The disturbance that amenorrhoea causes varies in individual women, but these figures allow pre-therapy counselling so that patients can be prepared for the amenorrhoea that ensues. The lower rate of amenorrhoea shown in this study makes a further case for the use of lower doses of danazol in the future. The occasional appearance of permanent voice change at high dosage supports the concept of the use of lower doses of danazol for benign indications (Wardle et al 1983). Sutton and O'Malley have shown that danazol at a dose of 50mgs per day gave an 83% response rate in an open study with only a 7% incidence of severe side effects (Sutton and O'Malley 1986).

A very large series of studies carried out in Hjorring in Denmark by Doberl, Tobiassen, Rasmussen and Rannevik entitled The Hjorring Project, describes the effects of varying doses of danazol from 400-100mgs daily in a well documented group of 124 patients with mastalgia (Tobiassen et al 1984, Doberl et al 1984). This group has shown results which are very close to those reported in this chapter, especially with regard to efficacy and side effects and have used very similar recording systems. Their general conclusions that danazol can be effectively used at lower doses than 200mgs for cyclical breast pain (Doberl et al 1984) is in agreement with the study described in this chapter.

Thus it appears that danazol at conventional dosage causes some side effects in 30% of women but only about 10%

will be unable to tolerate the drug, and about 70-80% of women with cyclical mastalgia will have some reduction in their breast symptoms.

CHAPTER FIVE

IMMUNOHISTOCHEMICAL STUDIES

5.1 INTRODUCTION

5.2 PATIENTS AND METHODS

5.3 RESULTS

5.4 DISCUSSION

5.1 INTRODUCTION

The two previous therapeutic studies had shown that both a prolactin lowering agent (bromocriptine) and an antigonadotrophin (danazol) effectively reduced the symptoms of breast pain and tenderness. The aetiology of mastalgia is unclear, as noted in the historical review, and so it is perhaps not surprising that two such different drugs are effective. Many animal studies have shown that oestrogen plays a central role in breast growth and development and even lactation in some species. Since danazol probably acts by indirectly opposing oestrogen (via ovarian suppression or possibly increase in free testosterone), the presence and activity of oestrogen receptors or oestrogen regulated processes in breast tissue taken from mastalgia patients would be of interest. Conventional dextran charcoal studies of oestrogen receptor (ER) or progesterone receptor (PR) content of benign breast tissue, have shown low values for ER and PR (Allegra et al 1979, Rosen et al 1975). Allegra et al found that 38% of benign breast biopsies were ER positive (>10 fmol/mg protein), but fibroadenomas were 55% positive (Allegra et al 1979). These low values are surprising as it might be assumed that the primary target organs for oestradiol, such as the breast and uterus, would contain high levels of ER. The explanation probably is that most benign breast biopsies contain low proportions of epithelium relative to stroma, unlike breast cancer tissue. Also the benign biopsies invariably come from premenopausal women who have a high

level of endogenous oestrogen and hence a high proportion of occupied receptors.

In support of the idea of a sampling problem in ER estimations on benign breast tissue, is the finding by a French group that highly cellular lesions such as fibroadenoma or ductal hyperplasia tend to have a higher ER content as measured by the dextran charcoal assay (Jacquemier et al 1982). This group showed an increasing level of ER, with an increase from bland fibrocystic disease (33% ER positive) up to ductal lobular hyperplasia (85% ER positive). Also, a further study of fibroadenomata showed an overall ER positive level of 41%, but the ER positivity appeared to be age related with higher ER values in the younger patients, again suggesting increased cellularity in the younger patient (Martin et al 1978). The study of Allegra et al also supports the finding of higher ER levels in more cellular tissue (Allegra et al 1979).

An alternative strategy is to use an immunohistochemical technique to examine oestrogen receptor, or a protein which is oestrogen regulated but is not bound to ER. Many oestrogen regulated proteins have been described including the 52K (Garcia et al 1984) and 24K (Ciocca et al 1982). The opportunity of examining the role of 24K protein by immunohistochemistry in mastalgia, became available to the author when this protein was being studied as part of a UICC Fellowship held at the University of Texas at San Antonio in 1982-83. 24K protein is a protein of

24,000 molecular weight, which is secreted into the medium when MCF7 cells are subjected to oestradiol rescue after nafoxidine arrest (Edwards et al 1980).Since oestradiol rescue after nafoxidine treatment is also the only reliable way of producing oestrogen stimulation of MCF7 cells,this suggested that the production of 24K protein is oestrogen regulated.The molecular weight of the protein was determined by SDS gel electrophoresis and these experiments also showed that a pronounced increase of radio-labelled material at a molecular weight of 24,000 was produced by oestrogen-treated MCF7 cells compared with control cells treated only with nafoxidine (Edwards et al 1980). Soon afterwards monoclonal antibodies (Mabs) were developed in San Antonio against the 24K protein (Ciocca et al 1982). Purified 24K protein was injected into BALB/c mice, and the mouse spleen cells were fused with NS-1 murine myeloma cells using polyethylene glycol; the resulting hybridoma supernatants were screened for binding to MCF7 human breast cancer cells. The resulting Mabs were then used in immunohistochemical studies of MCF7 xenografts grown in the nude mouse, and intense cytoplasmic staining was demonstrated (Ciocca et al 1982). Later work using the same Mab showed that 24K protein could be detected immunohistochemically in the normal target tissues for oestradiol such as uterus, oviduct, vagina and breast (Ciocca et al 1983a). The 24K protein was also found in oestrogen or progesterone receptor positive cell lines, but not in oestrogen receptor negative cell lines (Ciocca et al 1983b). When 24K levels were measured in human breast cancer cytosols using an enzyme-linked immunosorbent assay and compared

with ER and PR levels, a strong positive correlation was found with the main correlation coming through ER levels (Adams and McGuire 1985). These studies confirmed the strong correlation between the presence of ER and 24K protein. Further studies demonstrated the stability of the 24K Mab if stored undiluted at -20 C, and the ability of the Mab to give specific immunostaining at concentrations from 5 - 0.05 ugs/ml in formalin- fixed paraffin-embedded sections (Ciocca et al 1983b). These studies demonstrated the feasibility of using the 24K Mab on formalin-fixed human tissue to detect the presence of the oestrogen-regulated 24K protein by immunohistochemistry. This allowed the study of benign biopsy samples from women with mastalgia as a presenting complaint to be compared with samples taken from women with no history of mastalgia, to see if 24K protein was differentially expressed in the mastalgia group compared with the non-mastalgia group.

5.2 PATIENTS AND METHODS

(a) Patients

Seventy five benign biopsies were taken at random from the pathology files of the University Hospital of Wales Pathology Department,and 5 micron serial sections were cut from blocks which contained representative tissue. At least 5 sections were cut from each block to allow for loss of sections during processing and re-staining to verify the findings. The pathology report of each patient was verified to ensure that the biopsy was definitely benign. The sections were then stained immunohistochemically with anti-24K protein Mab using the method below, and the results read within one week of the staining procedure. When the whole series had been stained and read, the history of the patient was obtained from the Breast Clinic database. This records prospectively the presence or absence of breast pain as a presenting symptom, and allowed the patients to be divided into those presenting solely with a lump, and those presenting with a lump and mastalgia. The menstrual data of the patients and their oral contraceptive use was also obtained from the database. The intensity and distribution of the 24K immunostaining in the 2 groups was then compared.

(b) Immunohistochemical methods

An identical technique was used for all slides and is shown in table 5.1. The paraffin-embedded sections were first de-waxed in xylol and the xylol removed with alcohol. Then the tissue was immersed in methanol/hydrogen peroxide for thirty minutes to block endogenous tissue peroxidase. After a buffer wash, non-specific binding was reduced by incubation in normal horse serum for twenty minutes. The primary antibody (anti-24K), or a control antibody, were then added and left to incubate for three hours at room temperature. After the incubation period a buffer wash was followed by addition of the secondary antibody (biotinylated anti- mouse antibody) from the ABC Vectastain kit (Vector Laboratories, Burlingame, California, ref. no. PK - 4002) for thirty minutes. A further buffer wash was followed by addition of the Vectastain A and B reagents (avidin-biotin horseradish/peroxidase complex) for thirty minutes. This system is composed of an avidin-biotin complex which uses the high affinity of avidin for biotin, and gives a multiplication effect thus enhancing the sensitivity of the method (Hsu et al 1981). Avidin is a 68,000 molecular weight glycoprotein which has an extraordinarily high affinity for the small molecular weight vitamin, biotin. Because this affinity is over one million times higher than that of an antibody for its antigen, the binding of avidin to biotin is essentially irreversible. In addition to this high affinity, two other features enable the avidin/biotin system

to be effectively exploited: (1) Avidin has four binding sites for biotin, and (2) most proteins, including enzymes, can be conjugated with several molecules of biotin. These aspects provide the potential for macromolecular complexes to be formed between avidin and biotinylated antibodies. The complex attaches to the secondary biotinylated antibody, and the localisation of the secondary antibody is revealed by the addition of diaminobenzidine solution for five minutes - giving a brown colouration at the sites of attachment of the primary Mab (Fig 5.1). The slides were then counterstained with haematoxylin and dehydrated in an alcohol gradient into xylene for permanent mounting with Permount.

(c) Monoclonal antibodies and controls

All sections were stained at least twice on two separate occasions with anti-24K Mab. Each run consisted of a maximum of 20 slides, and negative and positive controls were included in each run. Positive controls were formalin-fixed paraffin-embedded sections of MCF7 xenografts grown in the nude mouse, which always gave a strong positive reaction with anti-24K antibodies (Fig 5.1). Two negative controls were employed in each run. One was an MCF7 xenograft slide which was passaged through the technique in table 5.1, except that the anti-24K primary Mab was omitted. The second negative control was an MCF7 xenograft section,which was passaged as in table 5.1, except that the primary Mab was a commercially available Mab of unrelated specificity (IgG mouse

monoclonal antibody against human IgE - supplied by Bethesda Research Labs, Gaithersburg, Maryland, USA).

Anti-24K Mab

This Mab was produced in Professor W McGuire's laboratory in San Antonio using established hybridoma techniques (Kohler and Milstein 1975). BALB/c mice were immunized with purified 24K protein obtained from cytosols of MCF7 cells grown in culture. The mouse spleen cells were fused with NS-1 murine myeloma cells using polyethylene glycol,and the hybridoma supernatants were screened 14 to 21 days after fusion. Positive hybridomas were cloned by limiting dilution, and the resulting monoclonal hybridomas were injected intraperitoneally into pristane-primed BALB/c mice. The antibodies obtained from the resulting ascites fluid were concentrated by ammonium sulphate precipitation giving a stock solution of anti-24K antibody with a concentration of 1 mg/ml. The stock solution was stored in aliquots at -20 C and thawed before use. Previous work had shown that undiluted ascites stock was stable for four months at -20 C (Ciocca et al 1983b). The anti-24K Mab was diluted to a working concentration of 5 ugs/ml as this had been shown to be the optimal working concentration for specific staining (Ciocca et al 1983b).

(d) Slide reading

Each tissue section was examined within one week of being processed, and the clinical details relating to the patient were unavailable at the time of slide reading. First, the positive control (anti 24K with MCF7 tissue xenograft) was checked to ensure that staining was present. If the positive control was negative the slides of that run were not read and the run was repeated. Then the negative controls were checked to ensure that they were negative. If the positive control stained positively and the two negative controls were unstained, the patient sample slides were then read. Only slides with definite ductal and lobular structures and other components of benign breast tissue such as apocrine metaplasia, fibroadenoma and cystic change were included in the study,as normal breast stroma was not stained by anti-24K Mab. The site of staining (focal or diffuse) was noted, and the intensity of the staining was rated as none (0),weak or moderate (+) or strong (++).

Table 5.1

Method for Immunohistochemical Staining of Paraffin Sections.

1. Circle tissue with diamond pen.

2. Deparaffinize and hydrate.

100% Xylol for 5 minutes.

100% Xylol for 5 minutes (2nd bath).

100% Ethanol for 3 minutes.

100% Ethanol for 3 minutes (2nd bath).

3. Incubate in H_2O_2/3% methanol for 30 minutes

180 ml Methanol

20 ml - Hydrogen peroxide 3%

4. Wash in PBS buffer (Phosphate Buffer 0.05M-pH 7-5) for 20 minutes.

5. Incubate in 3% normal horse serum for 20 minutes.

6. Blot excess serum from sections.

7. Incubate with primary Mab for 3 hours in moist chamber at room temperature.

8. Wash in PBS buffer for 10 minutes.

9. Incubate with secondary biotinylated antibody (Vector Laboratories - PK 4002) for 30 minutes.

10. Wash in PBS buffer for 10 minutes.

11. Incubate in ABC Vectastain reagents A and B for 30 minutes

PBS 2 ml

Reagent A - 12u1

Reagent B - 12u1

12. Wash in PBS buffer for ten minutes.

13. Incubate sections in peroxidase substrate solution for five minutes.

Peroxidase Substrate Solution

TRIS - 10 ml

DAB - 7 mg (diamino-benzidine)

H_2O_2 3% - 75u1

14. Wash sections in PBS buffer for five seconds.

15. Counterstain with Haemotoxylin for 30 seconds.

16. Wash in tap water for 10 minutes.

17. Wash in increasing concentrations of alcohol into Xylene

Ethanol 70% for three minutes

Ethanol 90% for three minutes

Ethanol 100% for three minutes

Ethanol (2nd wash) 100% for three minutes

Xylene 100% for five minutes

Xylene 100% for five minutes.

18. Mount sections with Permount.

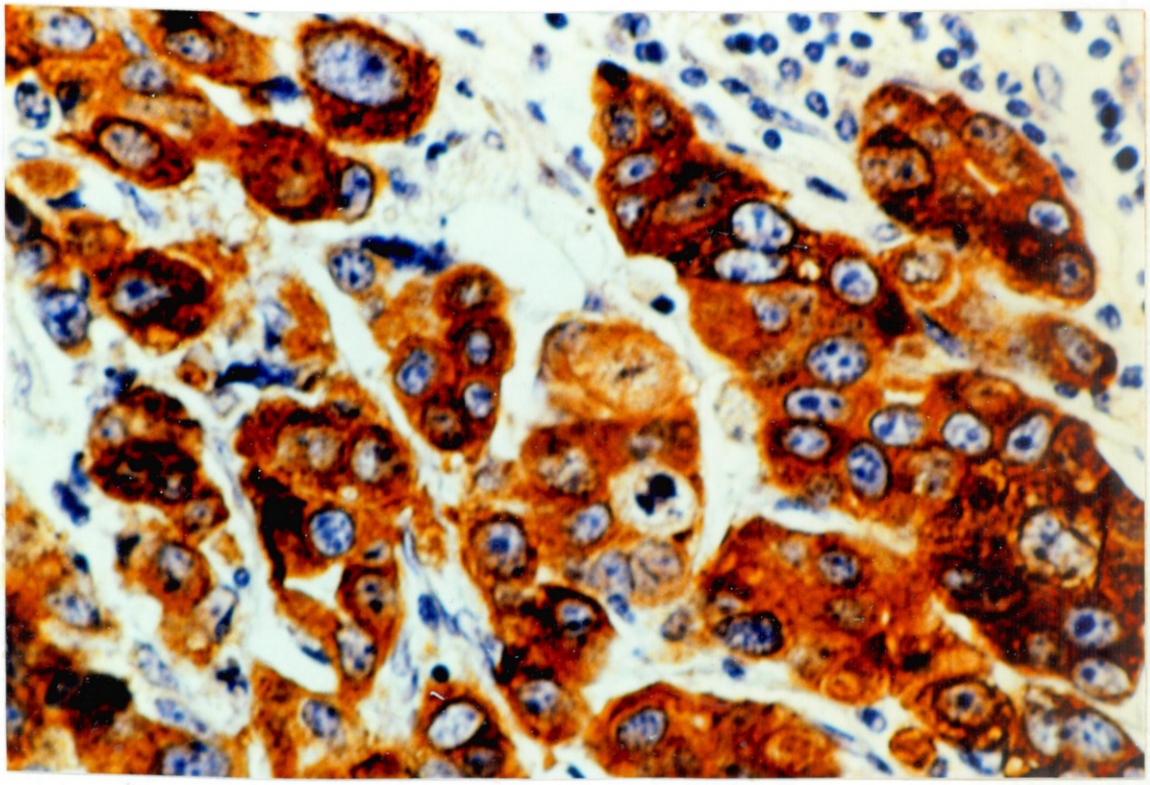


Figure 5.1 Positive control: MCF 7 xenograft paraffin-embedded, formalin-fixed and stained with anti-24K Mab by the method detailed in Table 5.1. The brown stain indicates the presence of 24K protein in the cytoplasm of the MCF 7 cells but no staining is present in the background mouse tissue x 400

5.3 RESULTS

Seventy five histological specimens were stained by 24K Mab using the immunohistochemical technique described in Table 5.1. All slides were read without knowledge of the symptoms of the patients. The indication for biopsy was a dominant breast lump in all cases except 2 who had nipple discharge. After the slide reading was complete,the computer database analysis showed that 20 of the patients had complained of mastalgia (Table 5.2) prior to the appearance of the lump, with a duration of mastalgia ranging from 1 month to 7 years (mean 14.5 months). The mean ages of the mastalgia and non-mastalgia groups were similar (42.6 $\pm$ 11 v 44.9 $\pm$ 3 years, mean $\pm$ SD. NS t test), and the proportion of current oral contraceptive users was almost identical (5/55 versus 2/20). The cycle length in those patients who were menstruating was similar (25.9 v 27.2 days), and the parity was identical in the 2 groups. Thus the 2 groups were well matched both in age and reproductive features.

Slide reading

The positive control of MCF7 cells with anti-24K Mab was always positive in all runs. A representative example is shown in fig 5.1. The technique produced an intense brown cytoplasmic stain with negative background stain. This strong positive result was seen if the xenograft MCF7 tissue or the loose cells were

Table 5.2 The Immunohistochemical Study

The 2 populations compared

	NO MASTALGIA	MASTALGIA	
	N=55	N=20	
AGE (Yrs)	44.9 ± 13	42.6 ± 11	NS t test
PILL USE Current	5/55	2/20	NS x^2 test
DURATION OF PAIN (Months)	N/A	14.5 ± 19.8	
LENGTH OF CYCLE (Days)	25.9 ± 3.5	27.2 ± 2.1	NS t test
PARITY	2.1 ± 1.7	2.1 ± 1.0	

Values are mean ± SD

used. The negative controls failed to show any intense staining (Fig 5.2) when the primary Mab was omitted or the anti-human IgE Mab was added. When positive staining occurred in duct or lobular tissue, it was located around both ducts and lobules (Fig 5.3) with diffuse cytoplasmic staining being the usual appearance. An example of moderate staining is shown in fig 5.3. Intense (++) staining was often seen in sections showing cystic changes with apocrine cyst linings (Fig 5.4). Again the stain was predominantly cytoplasmic, but the stroma never stained even when very intense staining was seen in adjacent epithelial cells (Fig 5.4). Negative staining was clearly discernible from positive staining as a comparison of fig 5.5 with fig 5.4 shows (both containing apocrine cysts). Occasionally, in positive staining areas of apocrine cysts, more intense staining was seen in the apical 'snouts' of the apocrine cells (Fig 5.6) suggesting higher concentration of 24K protein in these areas, but in the majority the stain was diffusely distributed throughout the cytoplasm.

The results were analysed and overall staining recorded as none, weak or strong (0, + or ++) and the results of this analysis are given in Table 5.3. There was a slightly higher negative staining rate in the non-mastalgia group, but this was not statistically significant (45% versus 40%). However, the proportion of weak staining was significantly higher in the mastalgia group (47 v 16%) and the proportion of strong staining higher in the non-mastalgia group (38 v 16%). These figures are statistically different($X^2 = 7.1$ $p < 0.01$ 1df). Since the strong

staining tended to occur in apocrine cysts, an analysis of the staining by tissue component was done, and this showed (Table 5.4) that ductolobular staining was 3 times higher in the mastalgia patients,but apocrine cyst staining was higher in the non-mastalgia group - a difference which is statistically significant ($X2 = 4.9$ $p< 0.05$ 1df). The proportions of sections containing apocrine cysts and ductolobular tissue were similar in mastalgia and non-mastalgia groups, thus suggesting that the higher proportion of strong staining in the non-mastalgia group was due to increased content of 24K protein in apocrine tissue in non-mastalgia patients.

The date of the last menstrual cycle prior to biopsy was known in 30 of the non-mastalgia patients and 13 of the mastalgia patients, and the day of the cycle on which the biopsy occurred was calculated. The proportions of positive and negative staining correlated with the day of the cycle are shown in figures 5.7 for mastalgia and 5.8 for non-mastalgia patients. The distribution of positive or negative staining in relation to the day of the cycle was similar in both groups (figs 5.7 and 5.8), but in the non-mastalgia group positive biopsies were more likely to come from mid-cycle or peri-menstrually, whereas the negative biopsies were evenly distributed over the whole menstrual cycle (fig 5.8). The mean cycle day in the negative and positive staining biopsies in the non-mastalgia patients was 15.2 ± 8.3 (mean $\pm$ SD) for positive staining and 11.4 ± 5.8 for negative. A similar trend was seen in the mastalgia patients, but these differences did not

reach statistical significance whether analysed by t test or X2 for proportions of luteal to follicular days (t=1.49 and X2=2.55).

These figures suggest that there is no increased expression of 24K protein at particular phases of the menstrual cycle although the mastalgia numbers are small.

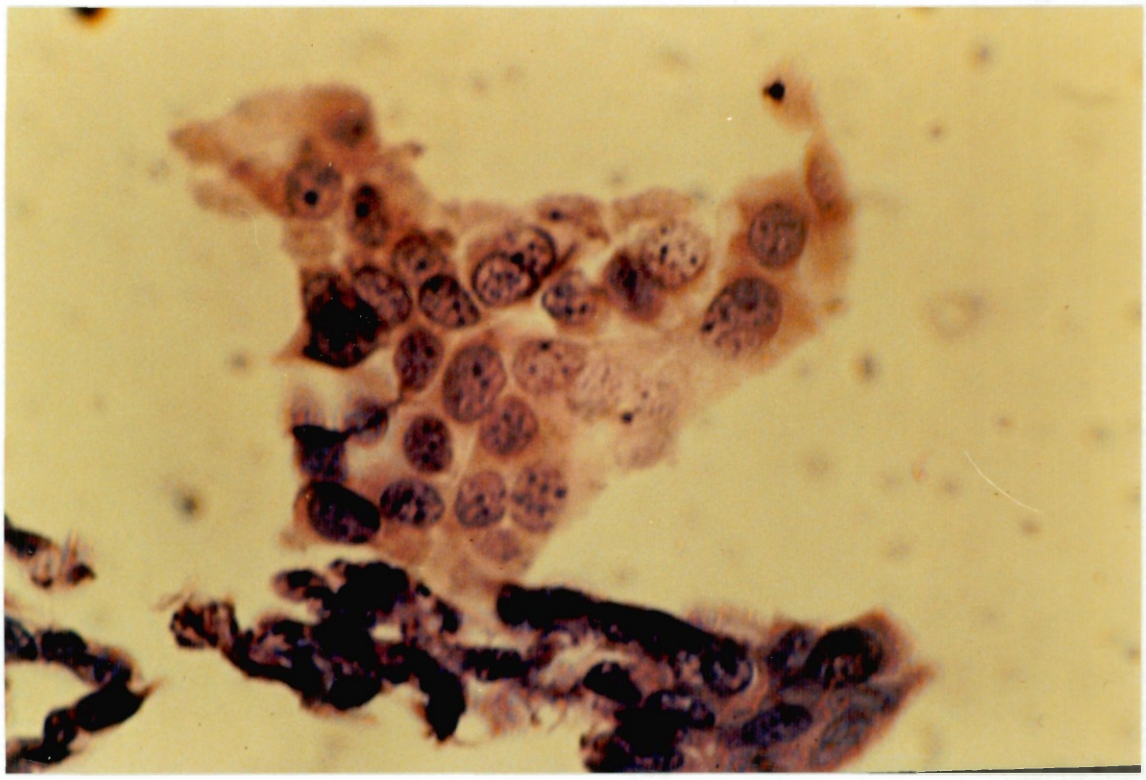


Figure 5.2 Negative control: MCF 7 cells in culture stained by the technique detailed in Table 5.1, but substituting mouse anti human I_gE antibody as the Mab. The absence of brown stain shows that the antibody has not bound to the cells x 400

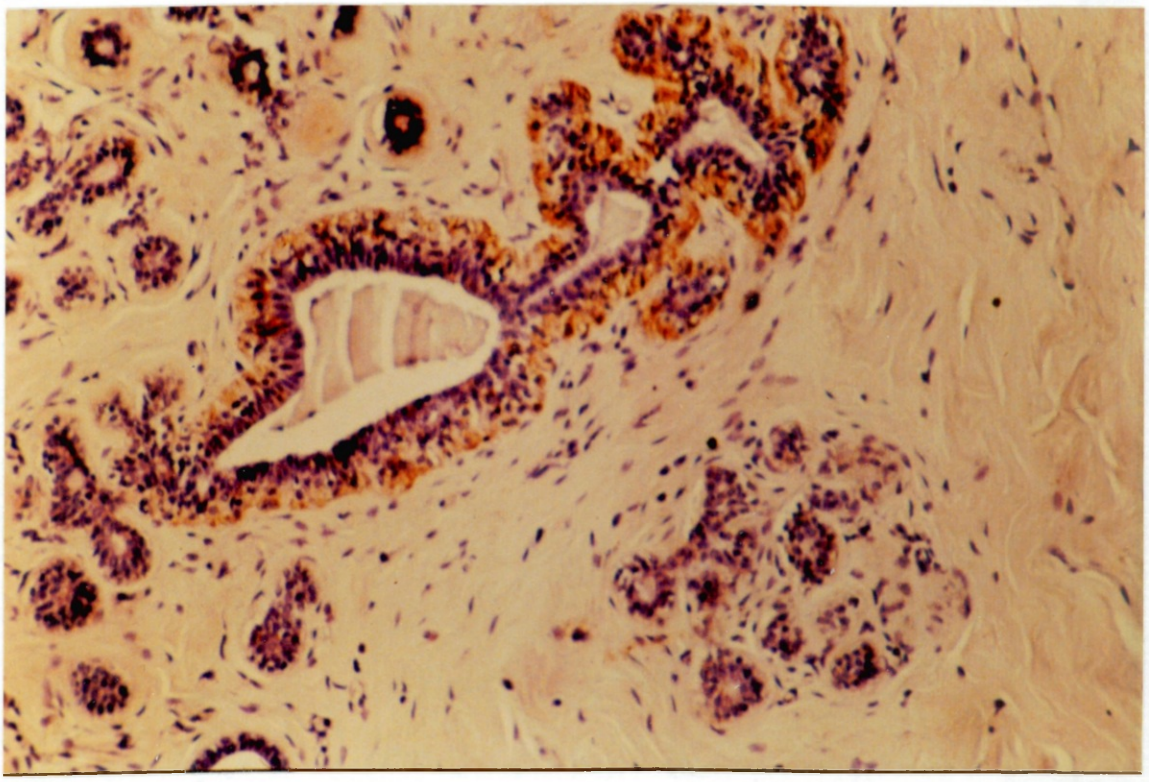


Figure 5.3 Section of human breast tissue showing moderate diffuse cytoplasmic staining in both lobules and ducts with the Anti-24K Mab at a concentration of 5µg/ml. Note the absence of stromal and luminal staining x 100

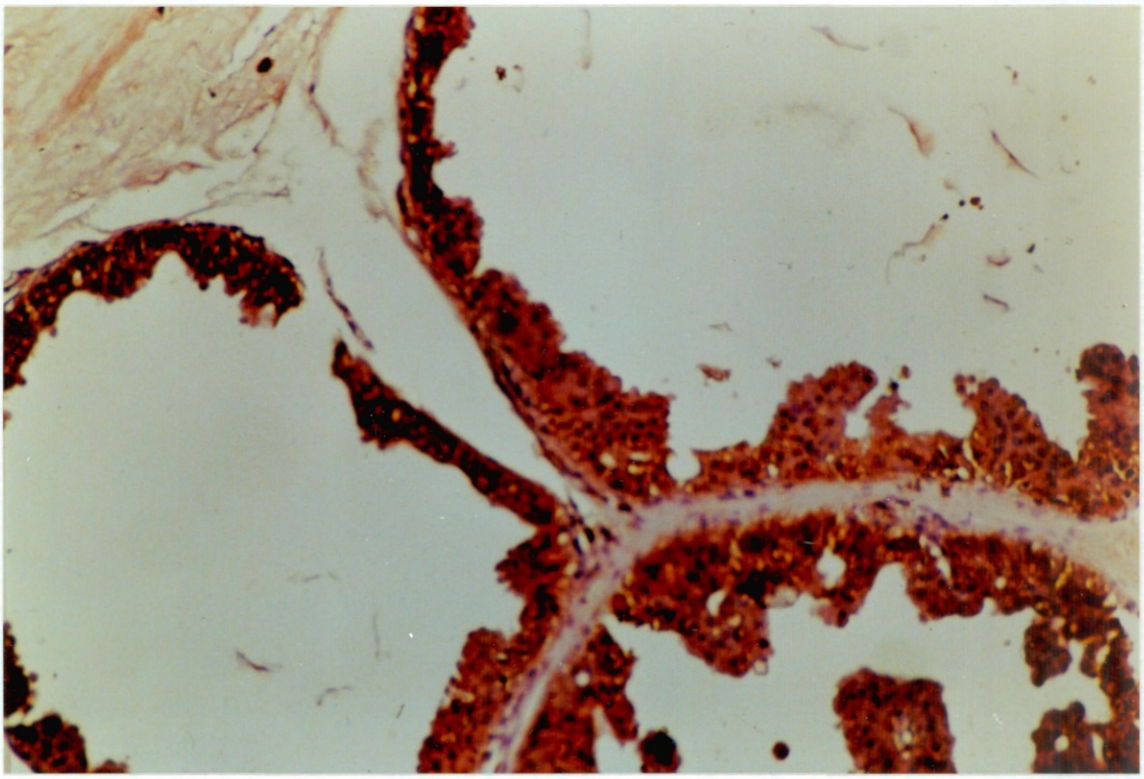


Figure 5.4 Section of human breast tissue showing intense cytoplasmic staining of apocrine lined breast cysts with the anti-24K Mab at a concentration of 5µg/ml. Note the absence of stromal or luminal stain x 100

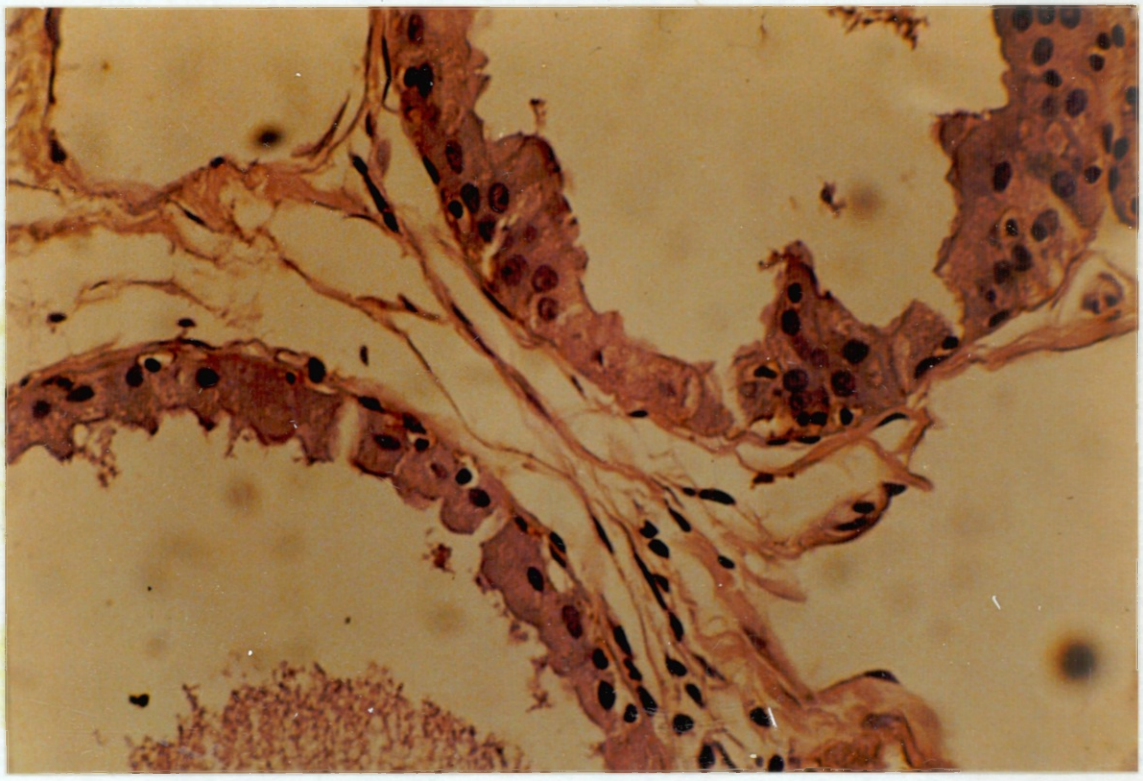


Figure 5.5 Section of human breast tissue showing apocrine lined breast cysts with no staining with the anti-24K Mab at a concentration of 5µg/ml x 250

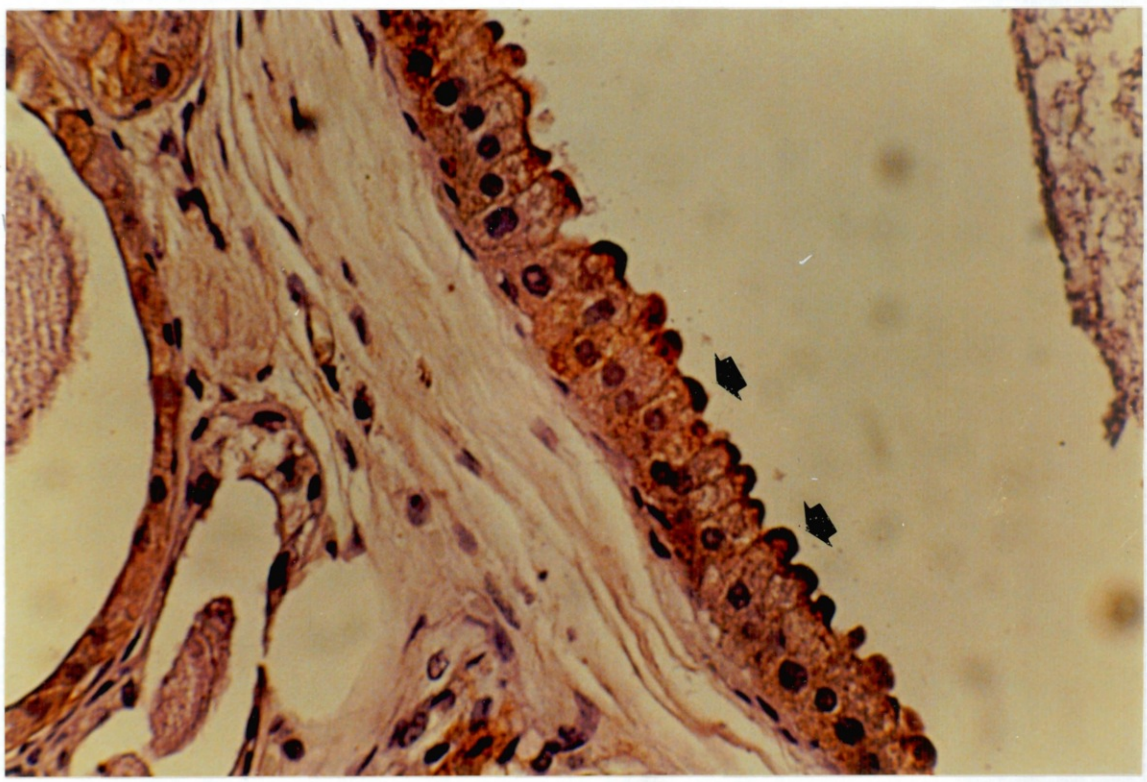


Figure 5.6 Section of human breast tissue showing an apocrine lined cyst stained with anti-24K Mab at a concentration of 5µg/ml.
Note the intense staining of the apical 'snouts' of the tall apocrine cells lining the cyst (arrowheads), and the absence of stromal or luminal staining x 400

Table 5.3 Overall staining results for 24K reactivity

	NO MASTALGIA N=55	MASTALGIA N=20
NO STAINING	25(45)	8(40)
WEAK POSITIVE	9(16)	9(47)
STRONG POSITIVE	21(38)	3(16)

No staining v weak and strong staining NS

Weak staining v strong staining $x^2 = 7.1$ $p < 0.01$ 1df
(No mastalgia v mastalgia)

Figures in parentheses are percentages

Table 5.4 24K Staining by tissue component

	NO MASTALGIA N=55	MASTALGIA N=20
DUCTOLOBULAR	5(9)	6(31)
APOCRINE METAPLASIA IN CYSTS	19(35)	5(26)
BOTH	6(11)	1(5)

Ductolobular v Apocrine/cysts or both $x^2 = 4.9$ $p < 0.05$ 1df

Figures in parentheses are percentages

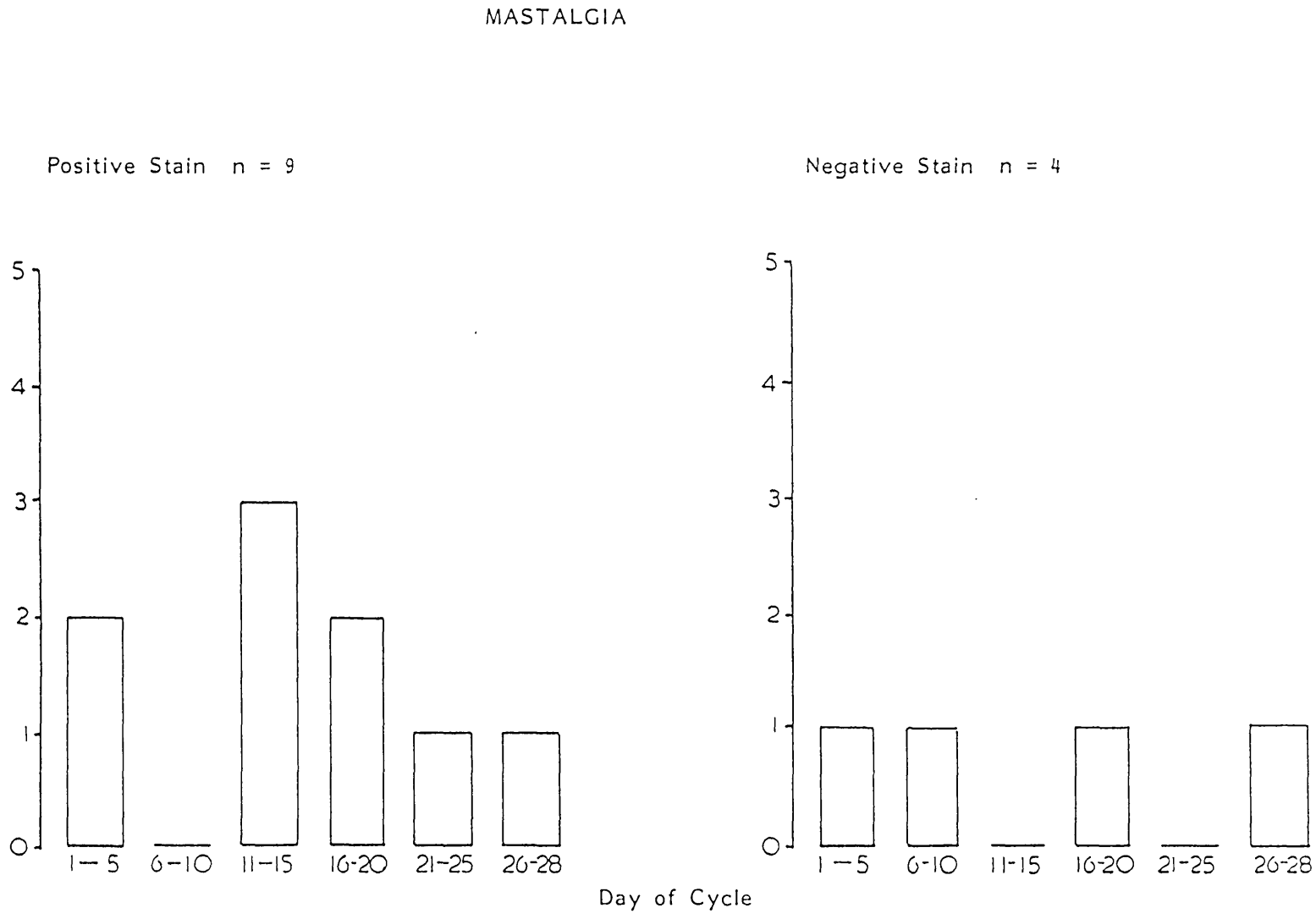


Figure 5.7 The day of the cycle of each biopsy plotted against positive and negative staining with 24K in the 13 patients with mastalgia.

NON-MASTALGIA

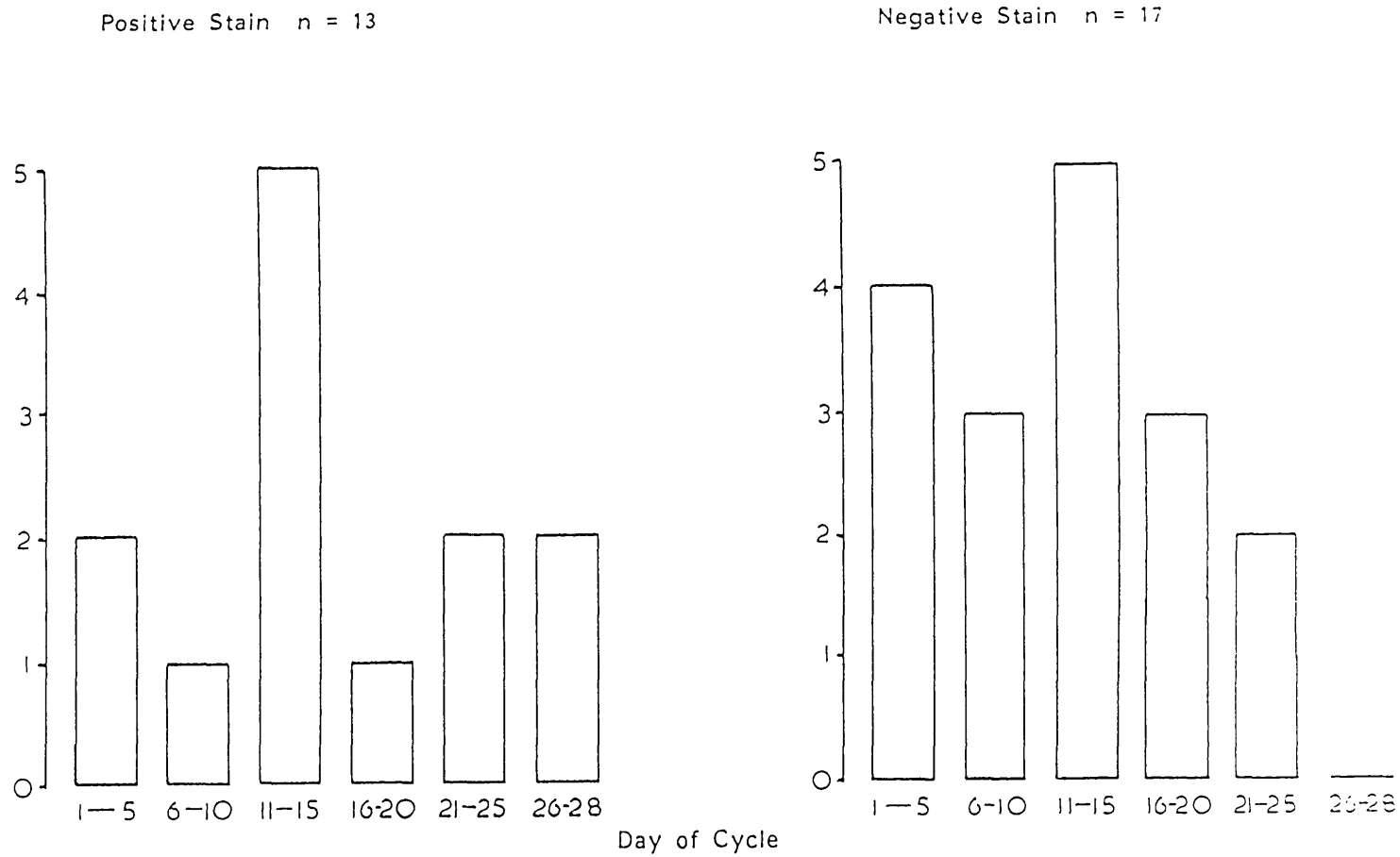


Figure 5.8 The day of the cycle of each biopsy plotted against positive and negative staining with 24K in the 30 patients without mastalgia.

5.4 DISCUSSION

Attempts to examine the biological properties of breast tissue in mastalgia have been very few, mainly due to ethical and technical difficulties. Watt-Boolsen et al conducted a similar study to the one described here, and looked at detailed histology and enzyme histochemistry in 32 mastalgia patients and 11 non mastalgia cases (Watt-Boolsen et al 1982). They found no differences between the mastalgia and non-mastalgia groups in either histological features or alkaline phosphatase activity in stromal fibroblasts.

The results of the immunohistochemical study using 24K Mab fail to support the suggestion that oestrogen expression at the target tissue level is increased in mastalgia patients. Indeed the non-mastalgia group showed a significantly higher intensity of staining, although this effect seemed to be more prominent in apocrine cysts rather than ductolobular tissue alone. When staining was confined solely to ductolobular tissue, the proportions of positive staining in the 2 groups were not significantly different, although this staining tended to be weaker than that seen in the apocrine tissue. Again the enhanced 24K activity seen in the apocrine cysts in the non-mastalgia group, would suggest increased metabolic activity in this group, as it has been shown that apocrine lined cysts are associated with high potassium levels in cyst fluid (Dixon et al 1983, Miller et

al 1983). The evidence from this study of 24K reactivity seems to suggest higher activity in the non-mastalgia patients.

Estimation of hormone action in human tissues is very difficult, expecially when that tissue is under the influence of several different primary hormones. The interaction between the various hormones and the second messengers generated intracellularly by those hormones, is a very complex system. However, monoclonal antibodies can accurately detect very small amounts of specific proteins using the highly sensitive immunoperoxidase system described in this chapter. Ciocca et al showed that 24K is an oestrogen regulated protein which measures oestrogen activity accurately as its level changes in endometrial tissue, in response to varying levels of oestrogen secreted throughout the menstrual cycle (Ciocca et al 1983c). This suggests that it should be a good marker of oestrogen activity within tissues, but in the study described in this chapter there appears to be no increased oestrogen expression in the breast tissue of mastalgia patients compared to a well matched group of non mastalgia patients. There is some evidence from this study that 24K protein may be secreted cyclically in the breast as the positive staining biopsies tended to come from the ovulatory or luteal phases of the cycle, but this change did not reach statistical significance. Ciocca et al did show a clear cyclical secretion of 24K protein in the endometrium (Ciocca et al 1983c). However, the distribution of staining by day of cycle appears to be similar in both mastalgia and non-mastalgia patients - thus

giving no support for an enhanced target tissue reactivity in mastalgia patients. Cyclical mitotic activity in benign breast tissue using measures of DNA synthesis, has been well documented by Masters et al (Masters et al 1977) and Anderson et al have shown cyclical activity of apoptosis or cell death (Anderson et al 1982). Thus it appears that benign breast tissue responds to hormonal fluxes as does the endometrium, but mastalgia patients have not yet been documented to have any enhanced hormonal responses at the target tissue level. Further work in this area is badly needed.

The higher staining seen in apocrine cysts is probably not important in the context of mastalgia, as it is known that the fluid derived from apocrine cysts has high levels of ions and steroids, suggesting that these cysts are highly metabolically active (Bradlow et al 1982). The ideal study would be a sequential sampling at different phases of the menstrual cycle, and on therapy for mastalgia, but obvious ethical constraints prevent such a study. However, the ability of Mabs to study small amounts of cellular material makes sampling by fine needle aspiration a practical possibility, and such studies may be feasible in the future. Indeed, Panahy et al recently showed an increase in progesterone receptors on danazol therapy in a group of mastalgia patients sampled sequentially by needle biopsy (Panahy et al 1987). In their study, danazol had little effect on oestrogen receptor levels, and they suggested that the beneficial action of danazol may be in part explained by induction of

progesterone receptors (Panahy et al 1987). Short term pre-operative bromocriptine gave an opposite effect in a French study (Peyrat et al 1984) They demonstrated a rise in oestrogen receptors but no effect on progesterone receptors, which was opposite to the findings of Panahy et al (Panahy et al 1987). Fentiman et al have recently shown that peri-operative bromocriptine reduced the DNA turnover (S phase fraction by flow cytometry) in breast cancer cells (Fentiman et al 1988). These studies suggest that both bromocriptine and danazol may be exerting a direct effect on breast epithelium which is independent of their effects on serum hormones. These studies used either open biopsy or needle biopsy techniques and are thus unsuitable for widespread use. These techniques were necessary because the steroid receptors were being measured by the dextran charcoal assay which requires large pieces of tissue. The advent of an immunohistochemical technique for ER estimation, should allow future studies of the hormone responsiveness of the breast tissue of mastalgia patients to be investigated, using material obtained by fine needle aspiration. A recent study of ER levels in normal breast tissue measured by immunohistochemistry of fine needle aspiration specimens, found that ER positivity was only seen in aspirates taken in the first half of the menstrual cycle (Markopoulos et al 1988).Such studies using Mabs directed at hormonally related antigens might clarify the mechanisms of action of drugs in mastalgia.

CHAPTER SIX

THE EVENING PRIMROSE OIL TRIAL

6.1. INTRODUCTION

6.2. PATIENTS AND TRIAL METHODS

6.3. RESULTS

6.4. DISCUSSION

6.1. INTRODUCTION

After the conclusion of the bromocriptine and danazol studies, it was clear that 2 effective agents were available, but side effects occurred in about 30% of patients on either of these agents. These problems and the need for mechanical contraception made these agents relatively unattractive therapy to all but the most severe cases of breast pain. In the search for an alternative therapy for the mild cases of breast pain, the literature on dietary influences in breast disease was examined. It is well known that there is a statistical correlation between 'economic development' of a country and the incidence of breast cancer, thus resulting in the high incidence of breast cancer in the USA and Western Europe, compared with Asia. This relationship is also present for dietary fat and breast cancer. However, there is no clear evidence of a positive correlation of obesity with benign breast disease, in fact the converse obtains as thin women have a higher incidence of biopsies, presumably because the physician can feel her breast nodularity more easily (Hislop and Elwood 1981).

In 1979, Horrobin published an hypothesis relating essential fatty acid deficiency to breast disease (Horrobin 1979). This work emanated from a careful study of the known actions of prolactin and the finding that prostalglandin synthesis markedly affected the action of prolactin on various tissues. The hypothesis suggested a cascade of events culminating in production

of prostaglandins, but the starting point for the cascade was the ingestion of various dietary essential fatty acids (Fig 6.1). Several links in the cascade were known to be affected by obesity and diabetes, both of which are linked epidemiologically to breast cancer. The essential fatty acids (EFAs) purported to be important in this hypothesis, are linoleic and y-linolenic acids, both of which are derived from dietary sources, especially plant oils. A high intake of saturated fat tends to block the conversion of linoleic to y-linolenic acid, thus resulting in a partial EFA deficiency. However, there was no concrete evidence of EFA deficiency in patients with benign breast disease. But Horrobin had noted by chance that several schizophrenic patients treated with evening primrose oil (EPO) had reported a resolution of breast pain and nodularity on this therapy (Horrobin-personal communication 1979).

Evening primrose oil is produced from the seeds of a biennial flower, the evening primrose (Oenothera biennis) which opens mainly at night (Fig 6.2). The oil is extracted from the seeds of this flower and is unique in having a very high level of y-linolenic acid (GLA). The oil used in this study was supplied by Efamol Ltd, and has been shown on analysis to contain 65-80% linoleic and 7-14% y-linolenic acids (Hudson 1984). This high level of GLA allows supplementation of the body stores by oral administration, and as GLA is one step further along the metabolic pathway of EFAs it gives the optimum boost to EFA metabolism. In view of the promising observation of reduction in breast

nodularity and the lack of side effects which had been noted, it was elected to formally test EPO in the treatment of painful nodularity of the breast in a controlled study. EPO provided an easy method of EFA supplementation as there are few natural sources of EFAs that are readily available. Human milk has been shown to contain some GLA (Gibson et al 1981), but the higher level of GLA in evening primrose oil is illustrated by the fact that 3 capsules of EPO contain the same amount of GLA as 1 litre of human milk.

A controlled trial of EPO in mastalgia has been carried out and is reported here. A pilot study carried out as a 2 centre study between Cardiff and Dundee using 6 capsules of EPO daily, had given conflicting results, although the assessment used Cardiff designed forms similar to those used in the previous trials in chapters 3 and 4. Several factors appeared to give rise to the differing results from the 2 centres. The criteria of pain severity were not strict and resulted in several patients with minimal symptoms being entered, and the Cardiff group had a higher proportion of patients who had failed a previous therapy. Thus the value, if any, of evening primrose oil was uncertain and a formal controlled study of previously untreated patients with severe breast pain was set up and is described in this chapter. The previous 2 centre study did however show that EPO was very well tolerated as there were very few dropouts on the preparation (Pashby et al 1981).

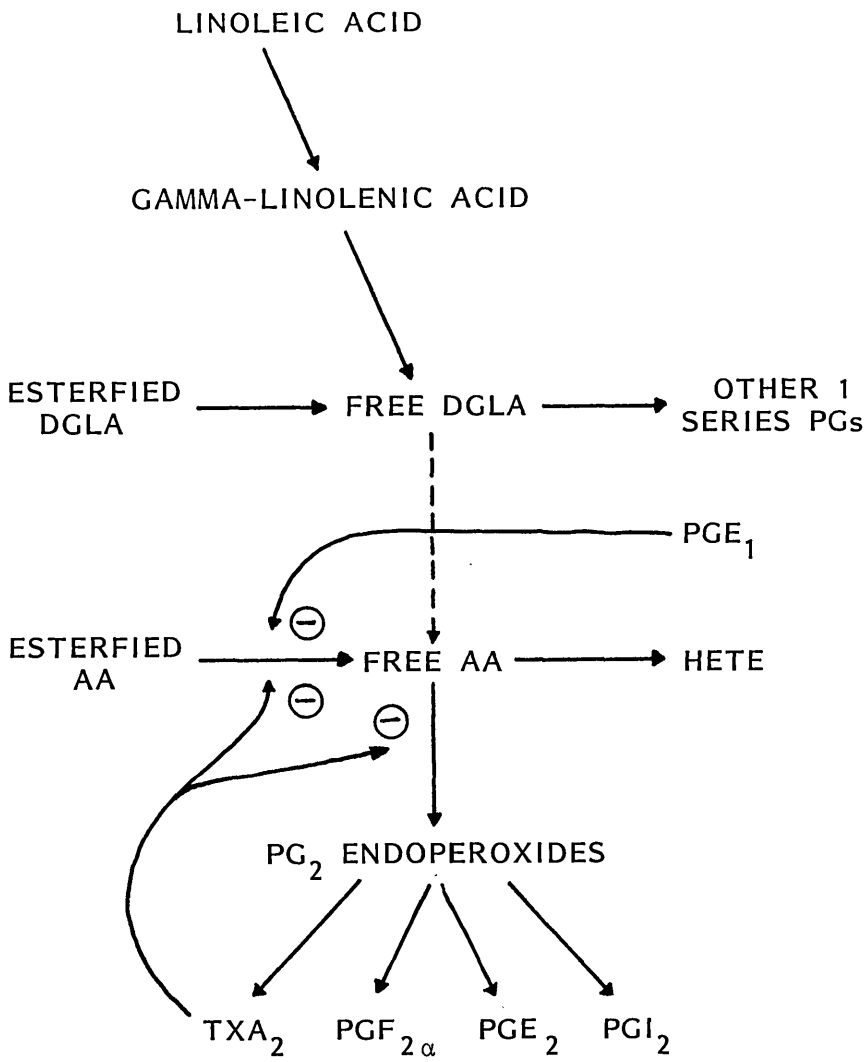


Figure 6.1 Metabolic pathways of prostaglandin synthesis (after Horrobin)



Figure 6.2 The Evening Primrose flower (Oenothera Biennis)

6.2. PATIENTS AND METHODS

(a) Patients.

This study was approved by the Medicines division of the DHSS and the Ethical Committee of the University Hospital of Wales. Patients over 18 years old were recruited as previously described, from the breast clinic of the University Hospital of Wales, after exclusion of breast cancer and with a main presenting complaint of mastalgia. Patients were first assessed by completion of pain charts as described in chapter 2, and those patients who complained that the mastalgia interfered with their everyday lives were then referred to the Mastalgia Clinic for consideration of entry into the trial. The intention of this study was to treat patients with cyclical mastalgia, and patients without a cyclical pattern on the pain chart were not entered. This did allow patients with a cyclical pattern who were post hysterectomy with ovarian conservation, to be entered. At the Mastalgia Clinic the pain charts were inspected, and if mastalgia of >7 days/month was present the patient was entered into the study. Patients who had previously failed on a hormonal treatment were not entered into the study. Women on the oral contraceptive were entered but no other hormone therapy was permissible. Patients with diabetes were not entered as this condition would inhibit the EFA pathways, notably the conversion of linoleic acid to y-linolenic acid (Horrobin 1979). If the patient was

admissible, full informed consent was obtained and after baseline VLA scores had been measured, the patient started on the randomised therapy. Forty six patients were entered into the study between April 1982 and January 1984.

(b) Methods

The trial structure was a randomised double blind format with a total length of therapy of 4 months (Fig 6.3). Patients were randomised to start on either 6 capsules of evening primrose oil (EPO) containing 500 mg of oil, or 6 identical gelatin capsules containing 500 mg of paraffin oil (placebo). These therapies were continued for 2 months, when the placebo group were switched to 6 capsules of EPO (unknown to the patient) for a further 2 months. The patients who had been randomised to start on EPO continued on EPO for the whole 4 months. The identity of the EPO -> EPO, and placebo -> EPO were unknown to both the patients and the clinician. This trial structure was necessary as a 2 month course of EPO would greatly elevate body stores of essential fatty acids, and a simple crossover to placebo would inevitably have a prolonged carry-over effect, thus making interpretation of the data difficult. The chosen format had the advantage of a comparison between a 2 month and 4 month course of EPO. Both groups of patients received Vitamin C tablets 50 mg daily throughout the 4 month period of therapy. This was thought to be necessary as it was known that low vitamin C levels

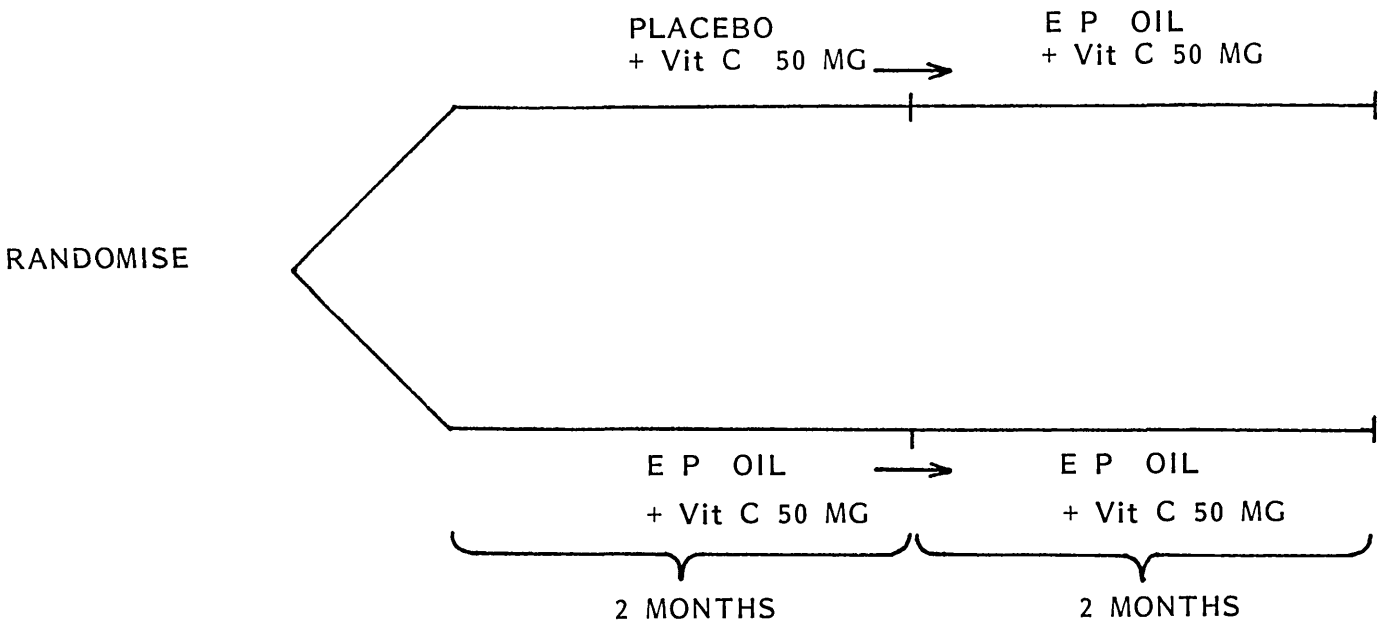


Figure 6.3 STRUCTURE OF THE EVENING PRIMROSE OIL TRIAL (EP OIL)

would inhibit the conversion pathways of EFAs (Carroll 1975).

(c) Assessment of response

Each patient was assessed by observation for 1 month on a pain chart prior to trial entry. The patients then continued to fill in a pain chart for the duration of the trial. At trial entry, which was arranged for the luteal phase of the cycle (day 20-28), a baseline VLA form (Fig 6.4), trial registration form (fig 6.5) and clinical assessment was completed (fig 6.6). Each patient was then seen monthly in the luteal phase of the cycle, and the VLA scores and clinical assessments were completed. The clinical assessment was made by physical examination of the breasts, and a score allocated for breast tenderness and nodularity on a scale from 1-3 where 1 = mild, 2 = moderate, and 3 = severe.

The VLA scale was a 100mm line as used in the previous trials. The VLA score was read off in millimetres from the zero mark (left hand end of the scale). These scores were all measured after the patient had completed the trial, and described the symptoms of pain, tenderness, heaviness, lumpiness for breast symptoms, and irritability, well being and effect of treatment for general symptoms.

At each visit the patient's weight was measured and enquiry was made of the regularity of the menstrual cycle and any side

effects that had been encountered. Capsule counts were made of the returned cartons of capsules at the end of each month.

(d) Blood samples.

A baseline venous blood sample was taken at the trial entry visit in the luteal phase of the cycle, and blood samples were taken at each monthly clinic visit. These samples were immediately separated by centrifugation and both serum and red cells were stored at -20 C for subsequent analysis of fatty acid levels. The EFA levels were measured by the Efamol Research Institute in Novia Scotia, Canada, at the end of the trial. The samples were identified by trial number and visit number and the assays were thus performed without knowledge of the treatment group the patient had been allocated. The results of the assays were then returned for analysis. The assay was performed by chloroform/methanol extraction of the serum and red cells, followed by thin layer chromatography. The methyl esters of the fatty acids were measured on a Hewlett-Packard 5880 gas chromatograph using a 6 foot column packed with 10% silar on chromosorb WAW 106/230 (Manku et al 1983).

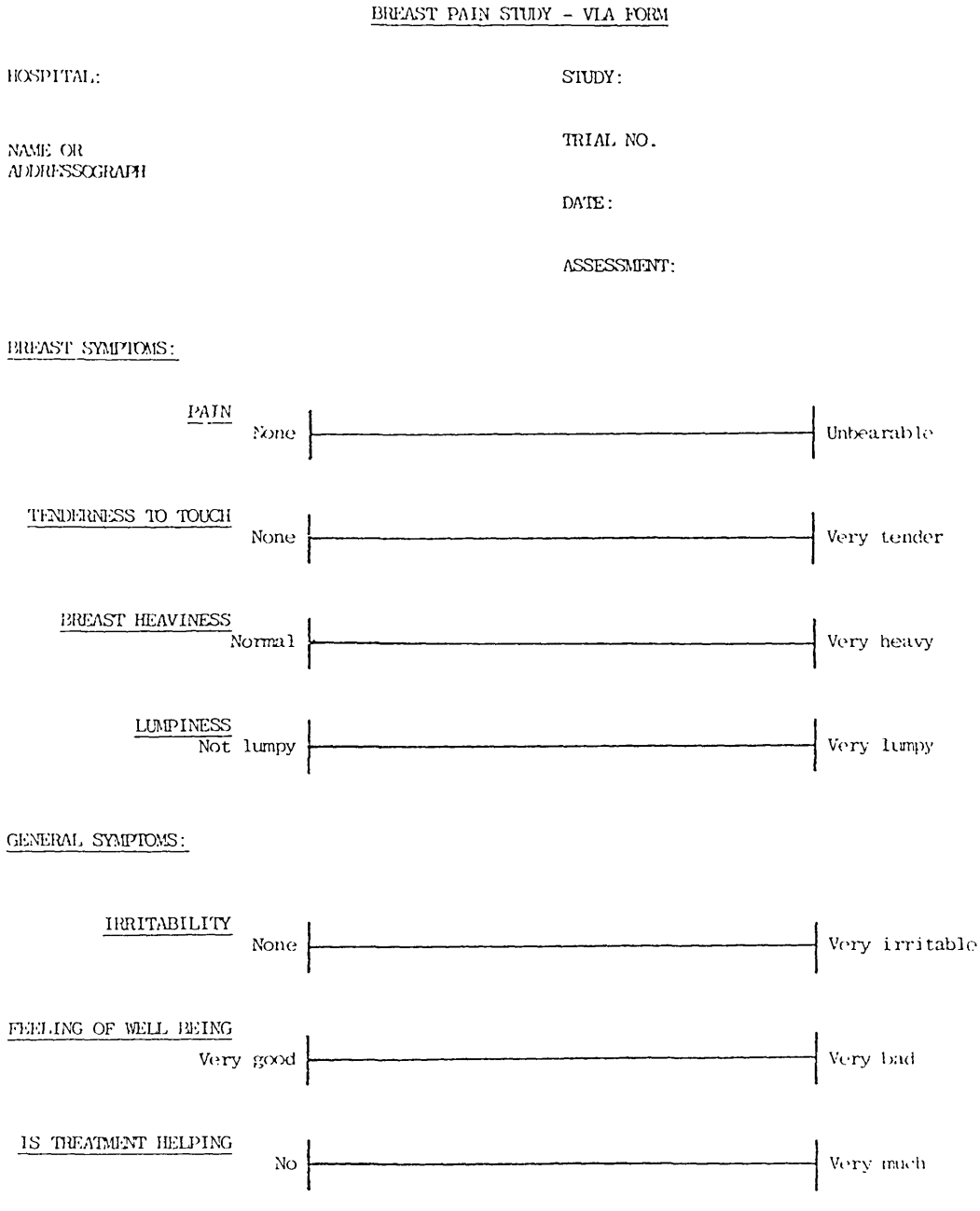


Figure 6.4 The VLA form used in the EPO trial

BREAST PAIN STUDY - REGISTRATION FORM

HOSPITAL

STUDY

TRIAL NO.

NAME AND D.O.B.
OR ADDRESSOGRAPH

DATE OF ENTRY

G.P. NAME AND ADDRESS:

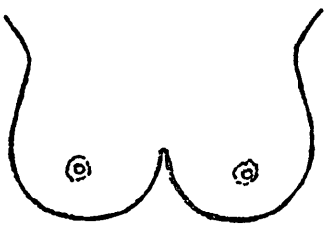
PAIN PATTERN

(a) Cyclical / Non cyclical irregular / Non cyclical continuous

(b) Duration

(c) Site

(d) Associated Nodularity Yes/No




Diffuse granularity


Local nodules

Menstrual and Reproductive History

Number of Pregnancies

LMP

Cycle Length

Oral Contraceptive History

NOTES (inc. blood tests, mammogram findings, etc.)

Figure 6.5 THE REGISTRATION FORM - EPO TRIAL

BREAST PAIN STUDY - CLINIC VISIT

HOSPITAL

STUDY:

NAME OR
ADDRESSOGRAPH

TRIAL NO.

DATE:

ASSESSMENT:
1,2,3, etc.

CLINICAL ASSESSMENT

a) TENDERNESS

0 = Not examined
1 = No tenderness
2 = Some tenderness
3 = Marked tenderness

(b) NODULARITY

0 = Not examined
1 = No nodularity
2 = Some nodularity
3 = Marked nodularity

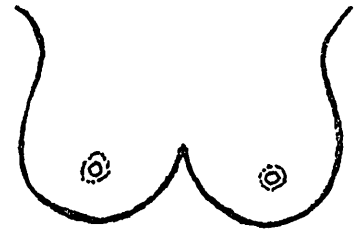
c) RESPONSE TO TREATMENT

0 = Not seen
1 = Asymptomatic
2 = Some improvement
3 = No improvement
4 = Worse

DESCRIPTIVE DIAGRAM

Diffuse granularity

Local nodules



Side Effects

Tablet count correct / incorrect

Weight

Notes - e.g. blood taken etc.

Menstrual cycle

Regular / Irregular / None

Figure 6.6 THE CLINICAL ASSESSMENT FORM - EPO TRIAL

(e) Statistical analysis.

The data from the clinican's scale of 1-3 were analysed by the Wilcoxon's matched pairs signed ranks test on a within patient basis and comparisons were made between the pretrial scores and the treatment periods.

The VLA scores were compared at the end of the 2 and 4 month periods, using the Wilcoxon's signed ranks test for both groups. This gives a magnitude and direction for each patient, and compares the EPO -> EPO group at times 0, 2 months and 4 months, and placebo -> EPO group at 0, end of placebo and end of EPO. Although the calculation of pretrial score versus 4 month score in the placebo -> EPO group was made, it is rather artificial as 2 treatment periods occurred in this group over the 4 months ie. 2 months placebo treatment followed by 2 months EPO treatment.

The pain chart data was extracted as days of pain/treatment month and compared using Student's t test. The EFA data was also analysed using Student's t test at similar time points.

6.3. RESULTS

A total of 46 patients were entered into the study, 21 patients were randomised to placebo as the first therapy, and 25 to EPO as the first therapy. The ages ranged from 20 to 52 years with a mean of 33 years - typical of a group of cyclical pain patients. The characteristics of the patients at entry are shown in Table 6.1. There were no significant differences between the EPO group and the placebo group with regard to age (32.9 yrs v. 33.2 yrs), duration of pain (2.8 yrs v. 4.4 yrs), presence of nodularity (19/24 v. 16/20), parity (2.3 v. 2.5) and length of menstrual cycle (27.9 v. 27.4 days) - thus confirming the random allocation to treatment. Two patients in the placebo group had had a prior hysterectomy, while one patient in the EPO group (no 3) had ceased menstruating. Although the intention was to treat only patients with a cyclical pattern of pain, it was considered on review of the pretrial pain charts, that 7 patients probably had a non-cyclical pattern, 4 in the EPO group and 3 in the placebo group. As these were evenly distributed between the groups and were a small number, they were analysed together with the cyclical patients.

Six patients dropped out during the trial (Table 6.2). Patient No. 20 developed a generalised itchy rash and treatment was discontinued in the first month of therapy. This patient was found to be taking EPO, but later challenge of vitamin C showed

Table 6.1 EPO trial - Patient characteristics on entry

	Means ± SD	
	EPO -> EPO group N=25	Placebo -> EPO group N=21
Age (yrs)	32.9 ± 7.5	33.2 ± 6.8
Weight (Kgs)	61.8 ± 11.3	60.5 ± 9.1
Parity	2.2 ± 1.7	2.5 ± 1.7
Cycle Length (days)	27.9 ± 2.1	27.4 ± 2.1
Pain Duration	2.8 ± 5.2	4.4 ± 6.0

Table 6.2 EPO trial dropouts and withdrawals

	Group	Dropout month	Reason
No 2	Placebo -> EPO	3	Failed to attend
No 20	EPO -> EPO	1	Rash on Vit C
No 23	Placebo -> EPO	2	Failed to attend
No 25	EPO -> EPO	1	Contracted hepatitis
No 28	EPO -> EPO	2	Failed to attend
No 30	EPO -> EPO	1	Did not wish treatment (asymptomatic)

that she developed an identical rash and so she is allergic to the vitamin C tablets given to both groups of patients. (A similar problem had been encountered in the pilot study in Dundee - P.E. Preece personal communication 1986). Patient no. 25 developed an intercurrent hepatitis and was withdrawn in the first month of therapy on EPO. Two further patients in the EPO group (nos 28 and 30) did not return to the clinic during the first and second months of treatment and were lost to follow up. Two patients in the placebo group also dropped out (nos 2 and 23), both at the end of the placebo treatment period and again were lost to follow up. Thus dropouts were about equal in the 2 groups, but were lower than had been found in the danazol and bromocriptine studies. Since all 6 patients had dropped out early in the trial and had no data to assess efficacy, they were excluded from the analysis of response to therapy, but are included in the analysis of tolerance and pretrial characteristics. This left 40 patients who had completed the full 4 months therapy and were evaluable for analysis, of which 21 were in the EPO -> EPO group and 19 in the placebo -> EPO group.

VLA results.

These were analysed by the Wilcoxon's signed ranks test, where each patient's pretrial score was compared with the 2 month (EPO or placebo) and 4 month scores (EPO end of 4/12 or EPO end of 2/12 treatment), and the difference between these time points are then given a sign (+ or -) and ranked in order of magnitude. The

sum of these ranks then gives 2 scores, one being positive and one negative. If there are no significant differences between the treatments, both scores will be fairly equal, but significant differences give an imbalance in the scores, the direction of which are given by the + or - sign. In these analyses an improvement in symptoms, i.e. a reduction in the VLA score, is denoted by a + sign.

The raw data used for the analyses at the 2 month and 4 month points, together with the pretrial value for each individual, are shown in Appendix V.

The raw VLA results (Appendix V, p405) do confirm that at entry most patients had very high scores for breast pain and tenderness, although it is clear that some patients describe their symptoms more as tenderness than as pain, as for instance, patient no. 15 who records a VLA score of 3 for pain but 94 for tenderness, and patient no.27 who gives a pain score of 5 and a tenderness score of 100. This confirms the necessity of using several different descriptions in VLA scales. It is also clear that the raw data shows some dramatic responses, where VLA scores reduce from around the maximum to the minimum e.g. patients nos. 1, 18, 38, 45, 48 and patients nos. 8 and 15, but most improvements are rather more modest. The mean values of each breast symptom at each time point are plotted as graphs in Fig 6.7 (pain), Fig 6.8 (tenderness), Fig 6.9 (heaviness) and Fig 6.10 (lumpiness), but the non-parametric Wilcoxon's test was used to

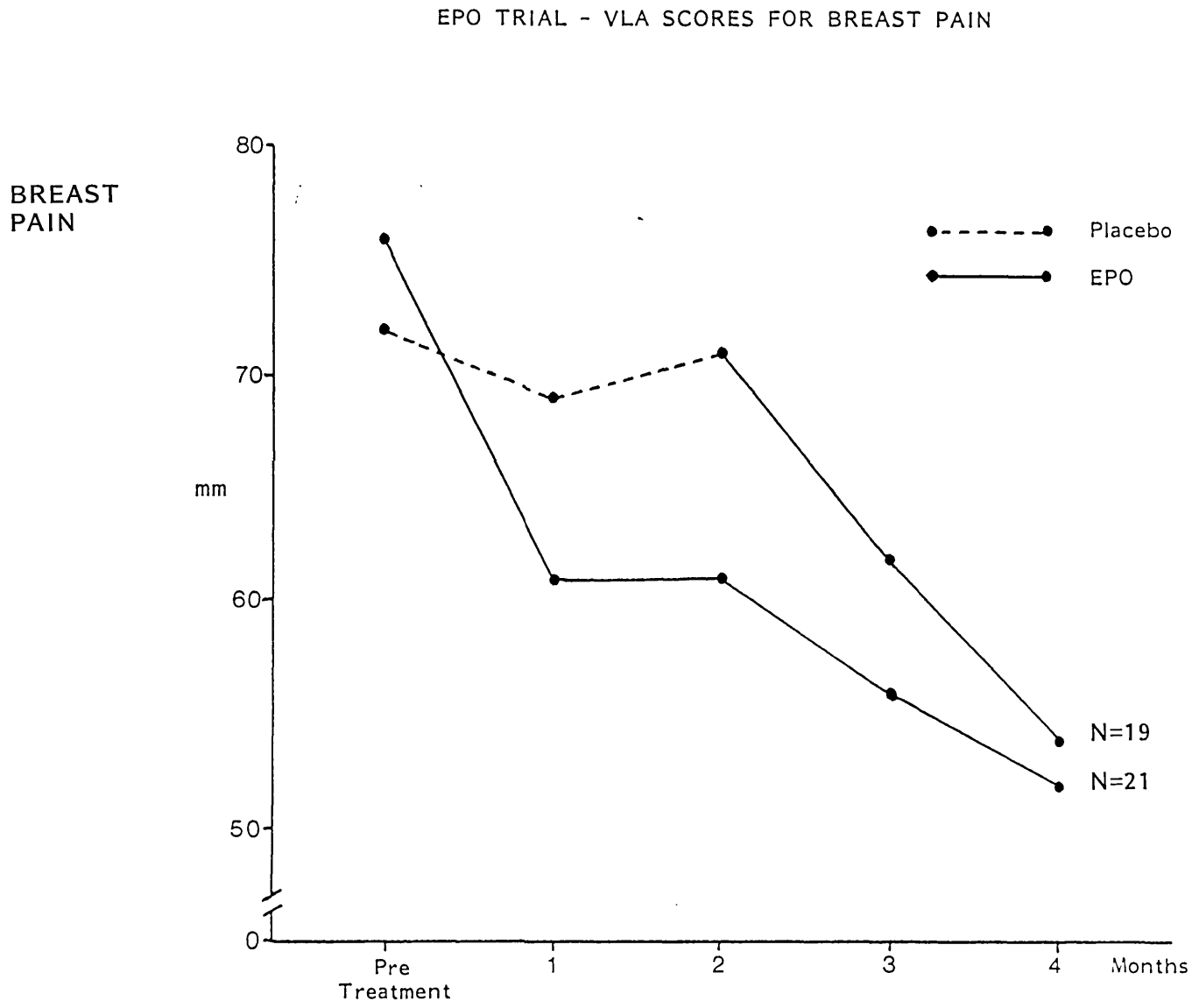


Figure 6.7 The mean VLA scores for breast pain in the 2 groups in the EPO trial

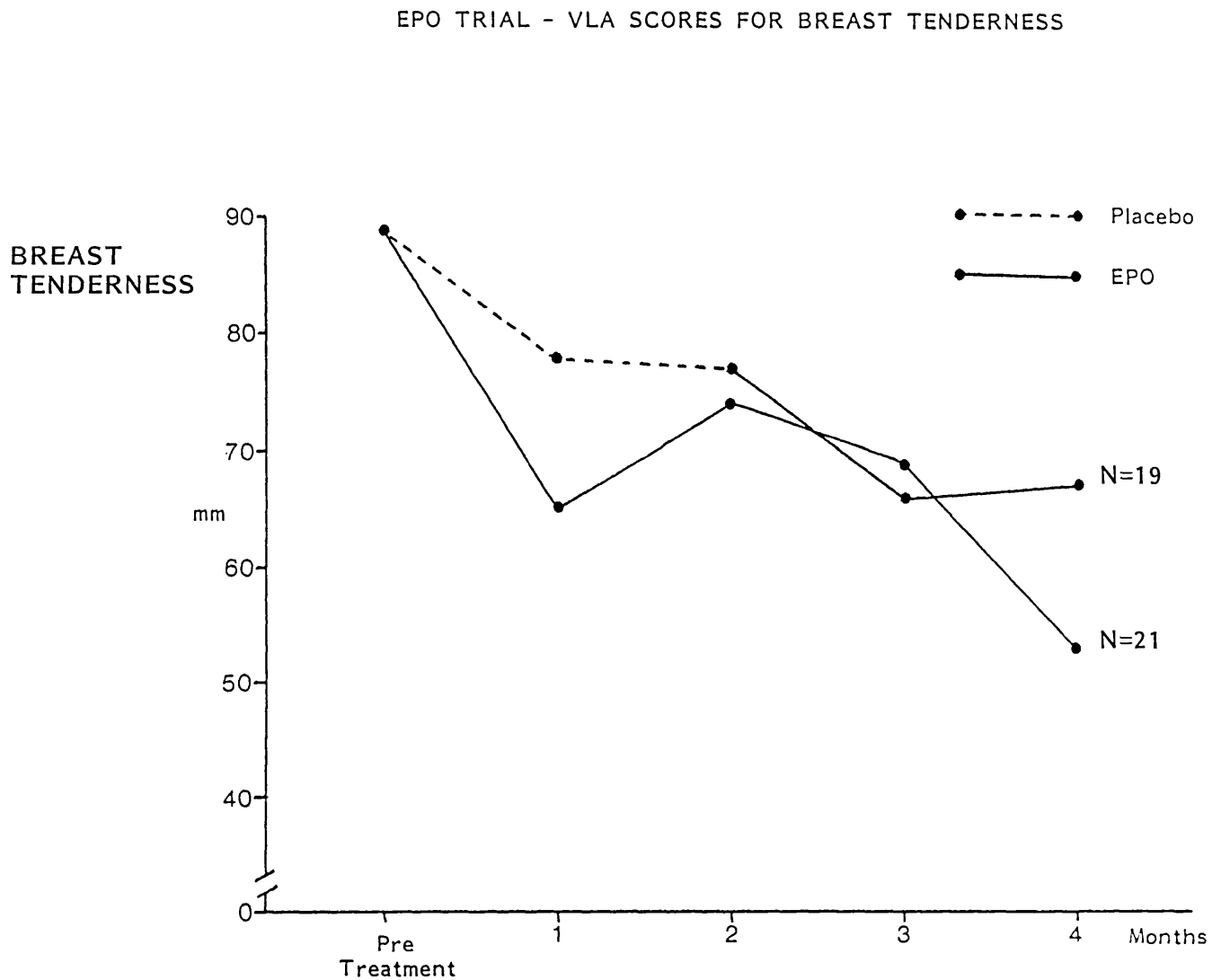


Figure 6.8 The mean VLA scores for breast tenderness in the 2 groups in the EPO trial

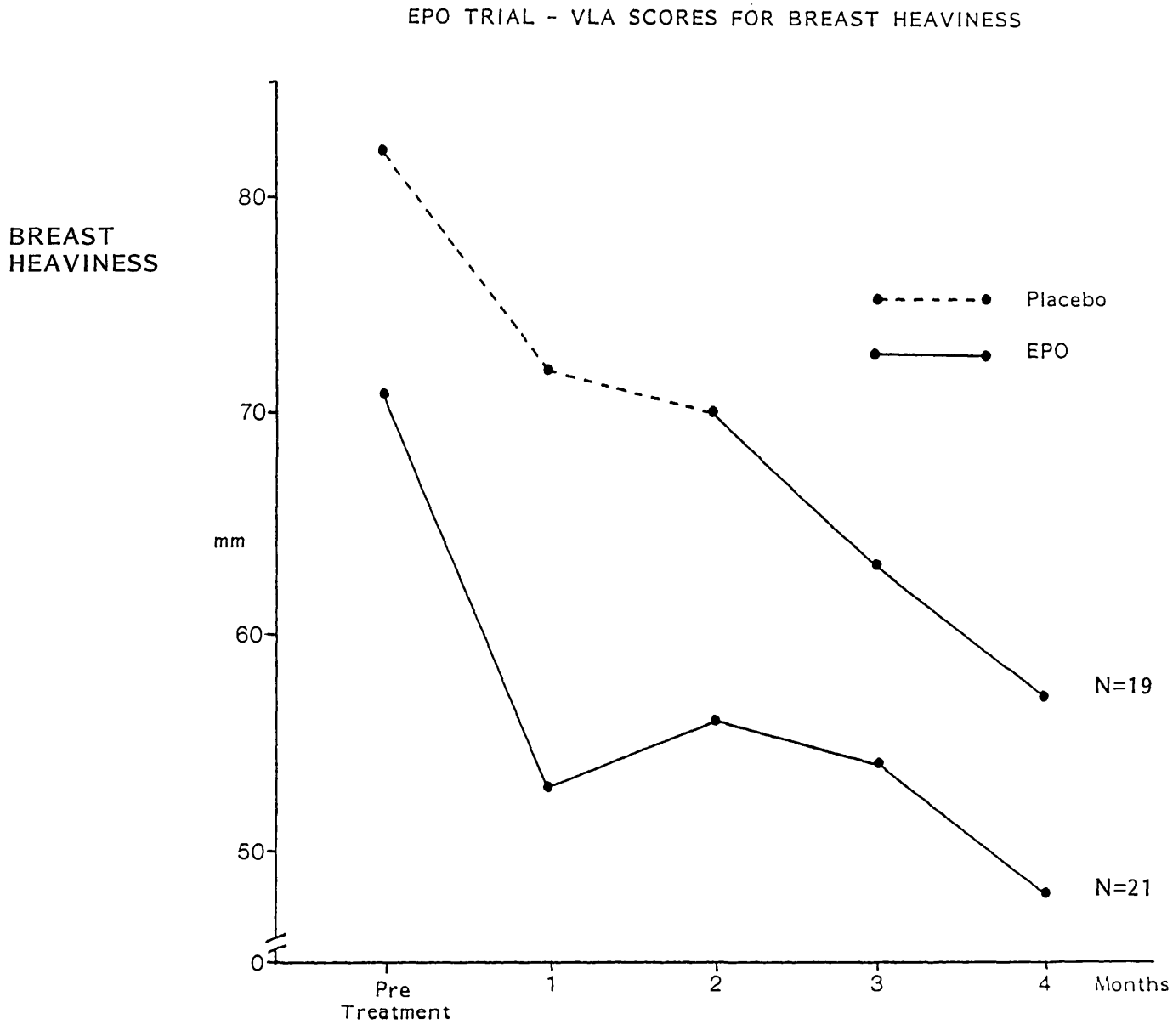


Figure 6.9 *The mean VLA scores for breast heaviness in the 2 groups in the EPO trial*

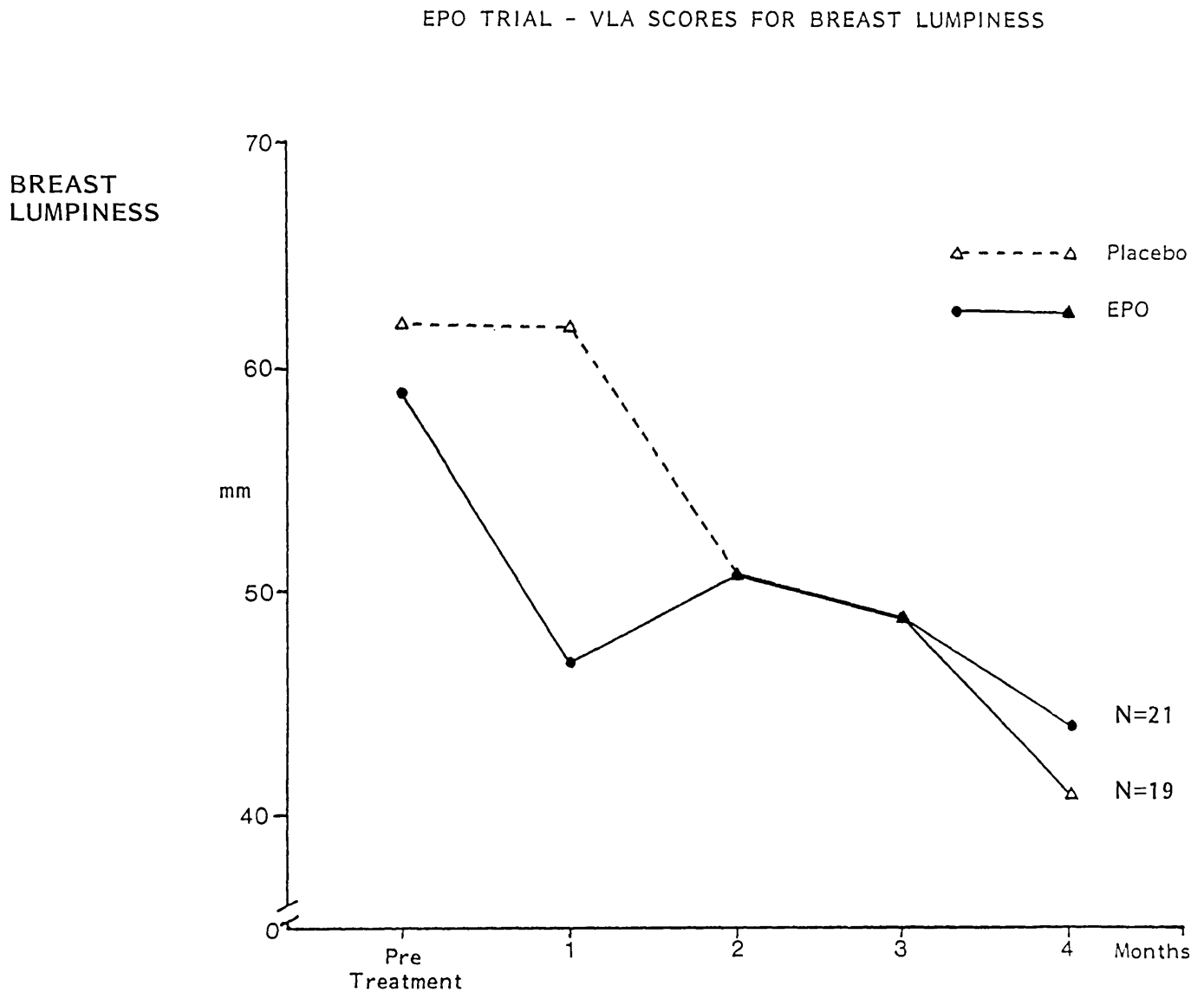


Figure 6.10 The mean VLA scores for breast lumpiness in the 2 groups in the EPO trial

assess statistical significance. Each symptom appears to respond to placebo, but the EPO treatment does produce a fall in VLA scores in all symptoms (i.e improvement in the symptoms), but as can be seen from the truncated vertical axes of the graphs, the falls are relatively modest with final VLA scores of approximately 50mm, suggesting the patients still had considerable symptoms.

The statistical analyses of the VLA results using Wilcoxon's test are shown in tables 6.3 and 6.4 for breast symptoms, and 6.5 and 6.6 for general symptoms.

The patients who took EPO for 4 months (table 6.3) showed no significant improvement in breast symptoms at the end of the 2 months treatment, although there is a suggestion of a positive effect as all the positive scores are greater than the negatives. After a further 2 months treatment with EPO, there were significant improvements in breast pain, tenderness and heaviness. Interestingly there was no reduction in lumpiness, with little change in the rank sum scores between month 2 and month 4. Thus it appeared that the effects of EPO took at least 4 months to become apparent.

In the placebo -> EPO group (Table 6.4) there were no significant improvements in any breast symptoms after 2 months placebo treatment. In these analyses 2 comparisons were made 0 v. 2 months i.e. pretrial v. placebo treatment, and 2 v. 4 months i.e. 2 months EPO treatment, compared with 2 months placebo

Table 6.3 VLA Analyses - Breast Symptoms EPO -> EPO group

	Rank Sums	
	Pre v 2 months	Pre v 4 months
Breast Pain	+142 -68	+168 -42 *
Breast Tenderness	+156 -53	+183 - 6.5†
Breast Heaviness	+137 -70	+149 -40 *
Breast Lumpiness	+111 -58	+117 -37

* $p < 0.02$

† $p < 0.001$

Wilcoxon's matched pairs signed ranks test (20 pairs)

Table 6.4 VLA Analyses - Breast Symptoms Placebo -> EPO Group

	Rank Sums	
	Pre v 2 months (Pretrial v placebo)	2 months v 4 months (Placebo v EPO)
Breast Pain	+ 76 -75	+127 -63
Breast Tenderness	+108 -44	+ 87 -33
Breast Heaviness	+ 97 -55	+ 78 -42
Breast Lumpiness	+ 83 -37	+ 79 -40

	Pretrial v 4 months (Pretrial v placebo + EPO)
Breast Pain	+111 -42
Breast Tenderness	+122 -31*
Breast Heaviness	+138.5 -32.5*
Breast Lumpiness	+128 -43

* $p < 0.05$ Wilcoxon's matched pairs signed ranks test (19 pairs)

treatment.

Comparison between the 0 and the 4 months scores showed that at 4 months a reduction in breast tenderness and heaviness just reached significance at the 5% level [Table 6.4, tenderness T = +122-31 (17 pairs) and heaviness T = +138.5-32.5 (18 pairs)]. This analysis shows that neither 2 months EPO alone nor 2 months placebo alone were able to improve the patient's breast symptoms, but the combination showed significant improvement in tenderness and heaviness.

The general symptoms of irritability, well being and the question of "is treatment helping?" were analysed similarly and the results are shown in tables 6.5 and 6.6. Table 6.5 shows that 4 months of EPO significantly improved irritability and well being in the EPO -> EPO group, but there was no improvement at the 2 months point. When the score at the end of the 2nd month was compared with that at the end of the 4th month, there were no differences in irritability or "is treatment helping?", but well being had significantly improved. In the placebo -> EPO group (table 6.6), irritability was significantly improved after 2 months placebo therapy but well being was unaffected. Neither irritability nor well being was significantly changed when the 2 months EPO treatment followed the placebo treatment. When the pretrial scores were compared with the score at the end of the 4 months (after 2 months placebo followed by 2 months EPO), only irritability remained improved, unlike the score after 4 months EPO (table 6.5), where both irritability and well being were

improved. When the end of placebo scores were compared with the end of EPO scores (table 6.6), there was a significant advantage to EPO in response to the question "is treatment helping?", suggesting that the patients felt better on EPO therapy.

Clinical Assessment

The results were assessed by the clinican using the simple scale 1-3 for breast tenderness, nodularity and whether the treatment was helping. The raw data are shown in appendix V but are summarised as means of each group in table 6.7.

As noted previously the 6 dropouts were excluded from this analysis, and missing data points in the other patients were allocated a value equal to that given at the preceeding month. The pretrial means for tenderness (2.3 for EPO ->EPO and 2.4 for Placebo -> EPO) and nodularity (2.0 for EPO -> EPO and 2.2 for Placebo -> EPO), were similar in both groups of patients, and confirmed the VLA data of high pretrial symptom levels. However, there was no significant change in symptoms at the end of month 2 although the tenderness score in the placebo group was lower than that of the EPO group. At the end of the 4th month, both groups showed a significant reduction in breast tenderness when compared with the pretrial means (1.6 v. 2.3 for the EPO -> EPO and 1.7 v. 2.4 for the placebo -> EPO). Breast nodularity scores were not significantly different in either group at either the 2 month or 4

Table 6.5 VLA Analyses - General Symptoms EPO -> EPO Group

	Rank Sums	
	Pretrial v end 2/12 EPO	Pretrial v end 4/12 EPO
Irritability	+131 -59	+171 - 39*
Well being	+114.5 -75.5	+158 -32†

	End 2/12 EPO v end 4/12 EPO
Treatment helping	+100.5 -70.5
Irritability	+156 -54
Well being	+145.5 -44.5*

* $p < 0.05$ Wilcoxon's matched pairs signed ranks test (20 pairs)

† $p < 0.01$ Wilcoxon's matched pairs signed ranks test (20 pairs)

Table 6.6 VLA Analyses - General Symptoms Placebo -> EPO Group

	Rank Sums	
	Pretrial v end placebo	End placebo v end EPO
Irritability	+136.5 -34.5*	+ 72 -33
Feeling of well being	+ 88 -65	+100 -36
Is treatment helping	N/A†	+ 34 -119[a]

	Pretrial v end EPO
Irritability	+135 -35.5*
Well being	+109.5 -43.5

* $p < 0.05$ Wilcoxon's matched pairs signed ranks test (19 pairs)

a $p < 0.05$ in favour of EPO Wilcoxon's matched pairs signed ranks test (19 pairs)

† no pretrial score possible

month point. The assessment of whether treatment was helping showed a slight fall in both groups, although neither was significantly improved.

In summary, the clinican detected significant improvement in breast tenderness in both groups at the 4 month point only, but a large part of the improvement in the placebo -> EPO group appeared to be due to placebo improvement at the end of the 2nd month.

Pain Charts

The data were expressed as total days of pain per month and means were compared by the Student's t test. Twenty nine complete pain charts were returned by the patients and were analysed. The data are shown in table 6.8. and Fig 6.11. Both groups of patients had equally severe mastalgia before therapy with a mean of 21.6 days of pain per month. This again shows the 2 groups of patients were well matched at randomisation. There appeared to be no effect on symptoms in the first 2 months of therapy of either EPO or placebo, as there was no significant change in the total number of painful days each month. However, in month 3 EPO produced a mean reduction of 5 days of pain and a further 3 days in month 4. At 4 months in the EPO -> EPO group, there was a significant reduction in days of pain per month with a halving of the number of painful days from 21.6 to 11.3 (Table 6.8).

In the group who started on placebo there was no beneficial

effect from 2 months placebo therapy, but on crossing over to EPO there was a slow reduction in days of pain in months 3 and 4 i.e. after 1 and 2 months treatment with EPO. This group gained a 7 day reduction in mean days of pain after 2 months treatment with EPO, compared with end of the placebo treatment period, but this did not reach statistical significance.

These figures closely agree with the VLA scores which showed that 2 months EPO treatment produced a non significant reduction in symptoms, whereas 4 months EPO treatment produces a highly significant reduction of mastalgia, with a halving of the days of pain each month. These data would suggest that EPO has a slower onset of action than either bromocriptine or danazol.

Weights were recorded at each clinic visit. The mean weights of both groups were identical (62 kg), with a range of 47.7 to 90.3 for the EPO -> EPO and 49.4 to 81 kg for the placebo -> EPO group. No significant changes in weight were detected in either group on placebo or EPO therapy (61 kg mean at month 4 for EPO -> EPO and 61 kg for the placebo -> EPO group).

No disturbance of menstrual cycle was noted either on placebo or EPO therapy.

Table 6.7 EPO trial - Clinicians Assessment at 0, 2, 4 months

Mean ± SD

GROUP	TENDERNESS			NODULARITY			TREATMENT HELPING		
	0	2	4	0	2	4	0	2	4
EPO -> EPO N=21	2.47±0.6	2.11±0.6	1.66±0.6*	2.04±0.6	2.09±0.7	1.76±0.7	N/A	2.62±0.8	2.28±0.7
Placebo -> EPO N=19	2.52±0.5	2.05±0.5	1.73±0.8*	2.26±0.6	1.95±0.6	1.84±0.7	N/A	2.63±0.7	2.15±0.9

* $p < 0.05$ v pretrial scores, Wilcoxon's matched pairs signed ranks test

Table 6.8 Daily pain chart scores - EPO Trial

	Mean days pain/month ± SD				
	Pre trial	1 Month	2 Months	3 Months	4 Months
EPO -> EPO N=16	22.1±4.4	19.1±6.0	19.2±7.1	14.8±4.5	10.7±5.5*
Placebo -> EPO N=13	22.5±4.5	21.2±6.2	23.4±5.5	18.0±7.3	16.0±8.8

* $p < 0.05$ v pre trial (Student's t test)

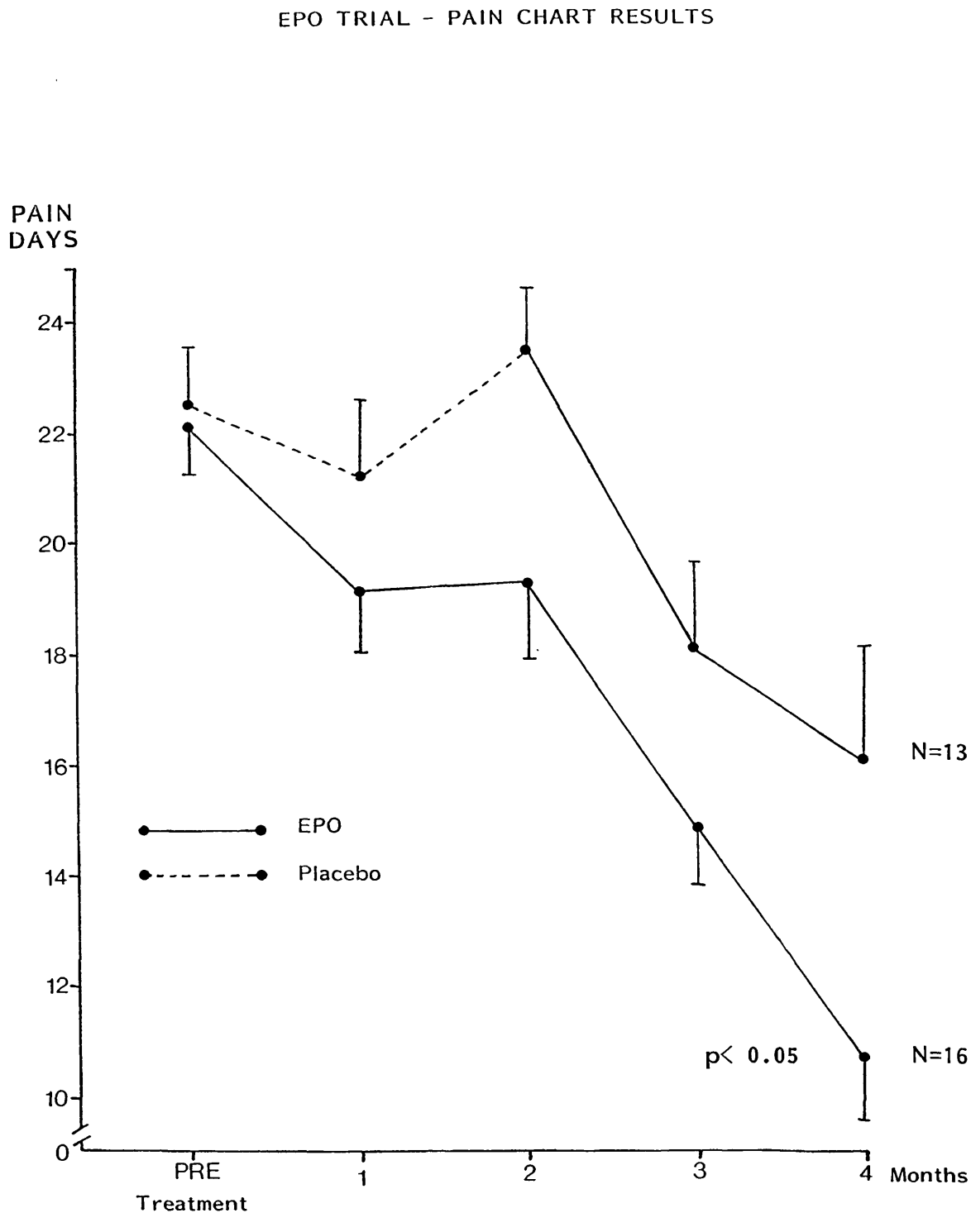


Figure 6.11 The pain chart results of the EPO trial shown as total days of pain/month (severe + mild). The error bars represent 1 standard deviation. The p value represents the comparison between pretrial values and 4 months EPO treatment using Student's t test.

Blood Results EPO Trial

The results of the fatty acid determinations were calculated as percentages of the phospholipid fraction extracted by chloroform / methanol from the plasma and red cells. The n-3 essential fatty acids (EFAs) derived from alpha-linolenic acid, and the n-6 EFAs derived from linoleic acid were both measured in the plasma phospholipid fraction and the red cell phospholipid fraction. Total plasma triglyceride and cholesterol was also measured in each group.

The results of the levels of cis-linolenic, linolenic, dihomo-gamma linolenic and arachidonic acids for the placebo to EPO group are shown in table 6.9. These are the principal EFAs which are postulated to be involved in the hypothesis of EFA deficiency suggested by Horrobin (Horrobin 1979). The results show a significant increase in dihomo-gamma linolenic and linolenic acids after 2 months treatment with EPO when compared with baseline levels, although smaller increases are seen after the first months EPO treatment (table 6.9). Placebo had no significant effect on any of the 4 principal EFAs. Table 6.10 gives the results for the EPO to EPO group and shows significant increases of dihomo-gamma linolenic acid in all four months of EPO treatment compared with baseline levels. The levels of cis-linoleic acid

were also significantly elevated after 3 months EPO treatment.

When the red cell EFA levels were analysed for the 11 patients in the placebo -> EPO group (table 6.11), and the 15 patients in the EPO -> EPO group (table 6.12) who had complete samples, a significant rise in arachidonic acid levels was found after 2 and 4 months treatment with EPO. Dihomo-gamma linolenic acid levels were increased after both 2 and 4 months treatment with EPO although these changes did not reach statistical significance. In summary, EPO treatment significantly elevated blood levels of dihomo-gamma linolenic and linoleic acids, and significantly elevated archidonic acid levels in red blood cells, whereas placebo had no effect.

Table 6.9 Fatty acid levels in total plasma phospholipids (% phospholipid fraction)

Placebo -> EPO Group (Means ± SD)

Fatty Acid	Baseline	1st Month Placebo	2nd Month Placebo	3rd Month EPO	4th Month EPO
18:2 ω 6 (cis-linoleic)	23.91±3.18	23.08±3.5	24.59±4.1	26.26±3.9	24.0±3.5
18:3 ω 3 (linolenic)	0.36±0.17	0.23±0.14	0.31±0.17	0.19±0.15*	0.20±0.15*
20:3 ω 6 (dihomo gamma linolenic)	2.36±0.71	2.26±0.81	2.45±0.51	2.85±0.73	3.22±0.53*
20:4 ω 6 (arachidonic)	7.82±1.47	8.09±1.89	8.62±1.92	8.56±1.82	8.91±1.15

* $p < 0.01$ compared with baseline (Students t test)

Table 6.10 Fatty acid levels in total plasma phospholipids (% phospholipid fraction)

EPO -> EPO Group (Means ± SD)

Fatty Acid	Baseline	1st Month EPO	2nd Month EPO	3rd Month EPO	4th Month EPO
18:2 ω 6 (cis-linoleic)	23.99±2.75	25.32±2.6	25.15±3.2	26.27±3.14 ↑	24.24±3.03
18:3 ω 3 (linolenic)	0.20±0.12	0.30±0.18 ↑	0.21±0.14	0.27±0.16	0.30±0.28
20:3 ω 6 (dihomo gamma linolenic)	2.28±0.42	3.36±0.52*	3.09±0.56*	3.28±0.69*	3.18±0.79*
20:4 ω 6 (arachidonic)	8.63±1.82	8.98±1.85	9.03±1.69	8.68±1.56	8.89±1.41

* $p < 0.001$ v. Baseline (Students t test)

↑ $p < 0.02$ v. Baseline (Students t test)

Table 6.11 Fatty acid levels in red blood cells - after 2 months treatment with EPO

	Placebo -> EPO group (N=11) Mean ± SD	
	Baseline	After 2/12 EPO
18:2ω6 (cis-linoleic)	18.75±4.50	20.87±4.08
18:3ω3 (linolenic)	Not detected	Not detected
18:3ω6 (gamma-linolenic)	Not detected	Not detected
20:3ω6 (dihomo gamma linolenic)	2.05±1.11	2.27±1.09
20:4ω6 (arachidonic)	9.57±3.69	13.69±5.18*

* $p < 0.01$ Student's t test

Table 6.12 Fatty acid levels in red blood cells - after 4 months treatment with EPO

EPO -> EPO (N=15)
Mean ± SD

	Baseline	After 4/12 EPO
18:2ω6 (cis-linoleic)	23.94±5.98	20.07±3.45
18:3ω3 (linolenic)	0.016±0.062	0.015±0.059
18:3ω6 (gamma-linolenic)	Not detected	Not detected
20.3ω6 (dihomo gamma linolenic)	2.00±1.13	2.49±1.09
20.4ω6 (arachidonic)	8.16±4.4	13.41±4.9*

* p <0.01 Student's t test v baseline

6.4 DISCUSSION

The results of the EPO study show that EPO at six capsules a day did significantly improve breast pain, tenderness and heaviness after 4 months treatment, but not after 2 months treatment. The patients entered into this study were comparable to those in the previous trials, as mean age and pain durations were similar at trial entry. The results do suggest a smaller response quantitatively on EPO therapy, compared with danazol or bromocriptine, as the VLA figures still showed mean symptom scores of about 50mm and the pain charts mean days of pain of 11 days even after 4 months therapy, while VLA means of around 20 mm were seen in the previous trials. However, it is difficult to draw any firm conclusions on comparisons between trials, as the statistical comparisons are made between patients before and after therapy. A trial comparing EPO with danazol or bromocriptine would be needed to answer this question. This trial did show placebo effects for both the clinician's assessments and the VLA assessment as in the other trials. The symptom of irritability on the VLA assessment was statistically improved after 2 months of placebo therapy (Table 6.6), but the clinician's assessment of tenderness and nodularity after placebo showed non significant changes (Table 6.7). As explained in Chapter 2, these findings are expected, as the patient will feel better for taking treatment prescribed and hence her mood or irritability will improve, and this improvement will be conveyed verbally and non-verbally to the assessing

clinician. These findings again emphasise the importance of having a placebo control in studies of mastalgia therapy.

The placebo effect on irritability was not reproduced in the breast symptoms (table 6.4), but neither were the breast symptoms improved after 2 months of EPO. It could be argued that a better test would have been a straight comparison between 4 months EPO treatment and 4 months placebo treatment, or even a 2 x 2 factorial design with EPO, EPO + Vitamin C, Vitamin C alone and placebo alone, but several practical problems would have occurred as patients would have to be told that they might receive only placebo treatment for 4 months. This tends to make patients either reluctant to enter a study or to drop out early. Also, larger numbers of patients would have been required with alternative designs which would have made it impractical to accrue the numbers needed, as only some 8% of patients attending the Cardiff Breast Clinic are referred to the Mastalgia Clinic, and only about 50% of these are eventually found to be eligible for treatment.

Comparison of the pretrial scores with the 4 month scores in the placebo ->EPO group (Tables 6.4 and 6.7) did show improvement in breast tenderness and heaviness, but the clinical significance of this is hard to interpret, as the patients had received 2 months placebo therapy followed by 2 months putatively active EPO treatment. It might be argued that 4 months placebo treatment alone would have also shown significant improvement, but this is refuted by the pain chart data (Table 6.8) which only shows

significant improvement in the EPO -> EPO group. Also the symptomatic improvements on EPO treatment were paralleled by increases in DGLA and red cell arachidonate levels.In contrast, the daily pain chart results (Table 6.8), did not show a placebo effect in the group who were randomised to start on placebo therapy, in fact, the mean days of pain increased slightly. This finding, which is unexpected, but probably correct as it also occurred in the pain chart data in the danazol trial, suggests that the data obtained from the pain chart may reflect the therapeutic situation more accurately. The rather modest gains produced by EPO treatment were well demonstrated by the pain chart, as 2 months of EPO treatment following placebo therapy produced a fall of only 5 mean days of pain compared to the pretrial values in the placebo to EPO group, and a fall of only 2 mean days after 2 months EPO therapy in the EPO to EPO group. The greatest improvement was seen after 4 months treatment with EPO, when the mean days of pain fell to 11.3 days, a gain of 10 days compared with the pretrial value. However the EPO -> EPO group did show a progressive decline in symptoms throughout the treatment period, unlike the placebo -> EPO group. Thus the pain chart, although not demonstrating a placebo effect, does agree closely with the VLA data at the 4 month treatment point. These findings would not have been demonstrated had the conventional double limbed crossover trial structure been used for this study. However, studies of EPO in other conditions, and the proposed mechanism of action did suggest that the effects would be seen more slowly than with danazol or bromocriptine. The results of

this trial show that the speed of onset of the therapeutic effects of EPO is indeed quite slow, and suggest that a minimum of 4 months treatment should be given before non-response is accepted. More realistically, a 6 months course should be used as the VLA graphs were showing a continuing improvement right up to the 4 month treatment point. These long treatment courses using EPO would be perfectly feasible as the agent does have remarkably few side effects, as might be predicted for a natural substance. Only 6 patients dropped out of the trial (Table 6.2) and 1 was taking placebo at the time, while 1 suffered an attack of hepatitis, so in reality only 4 of 46 patients dropped out on EPO therapy (9%), a rate which is much lower than bromocriptine or danazol. One of the dropouts was later found to be allergic to the Vitamin C tablets used in the trial, and thus the true dropout rate due to side effects of EPO was 7%. The general lack of side effects is a major advantage of EPO therapy, and the fact that it is a natural plant oil and not a 'drug' is also very attractive to patients. It had no demonstrable effects on weight or the menstrual cycle, further points which are attractive to patients considering therapy for their mastalgia. Another advantage is that patients do not have to stop their oral contraceptive while on EPO therapy.

There are few comparative data in the literature of EPO therapy in mastalgia. The findings of this trial agree closely with the pilot studies of EPO in mastalgia carried out in Dundee and Cardiff in respect of low side effects and modest improvements

on EPO (Pashby et al 1981, Preece 1982). Currently Vitamin C is not being given with EPO, as it appears to be unnecessary for the therapeutic effects of EPO, and as shown in this trial, it increases the incidence of side effects.

The blood results were extremely interesting as EPO had significant effects on the levels of fatty acids in the phospholipid fraction of plasma and red cells. The plasma phospholipid content of dihomo-gamma linolenic acid (DGLA) was significantly elevated in patients taking EPO for 2 or 4 months. These results do suggest an active metabolic effect of EPO as the oil contains some 7 - 14% of gamma-linolenic acid, the immediate precursor of DGLA, which was shown to be significantly increased by EPO therapy. It has also emerged that the baseline EFA values of the mastalgia patients studied in Cardiff and Dundee, are grossly abnormal compared with the levels of Cardiff controls (Table 6.13) Previous studies of EFA levels in normal controls have shown little variation with age or sex in the EFA levels in the plasma and red cell phospholipid fractions (Manku et al 1983, Holman et al 1979). It is thought that the phospholipid fraction levels in the red cells represent the long term dietary intake of EFAs (Faquhar and Ahrens 1963). The values for DGLA were significantly lower in the Cardiff and Dundee cyclical mastalgia patients, but the most striking difference was in the arachidonic acid levels which were very low compared with the controls (by at least 2 standard deviations). The cyclical mastalgia cases from Cardiff and Dundee show strikingly similar EFA abnormalities, in

Table 6.13 Baseline Fatty acid levels in plasma

(expressed as % phospholipid fraction mean ± SD)

	Cardiff Controls N=33	Cardiff Mastalgia N=46	Dundee Mastalgia N=17	PMS Cardiff N=54
18:2n-6 (cis-linoleic)	26.63±3.3	23.95±2.9	24.99±2.0	25.14±3.5
18:3n-3 (linolenic)	0.22±0.2	0.28±0.15	0.31±0.2	0.33±0.3
20:3n-6 (dihomo-gamma linolenic)	2.88±0.7	2.32±0.5*	2.29±0.5*	2.67±0.7
20:4n-6 (arachidonic)	11.15±1.5	8.3±1.9†	8.07±1.3†	9.0±3.6†

* $p < 0.05$)
) Student's t test v controls
† $p < 0.01$)

Cardiff controls = age matched women with no breast pain

that the arachidonic and DGLA levels are low compared with controls. Baseline EFA plasma levels have also been measured in patients with the premenstrual syndrome, and these also show abnormally low levels of arachidonic acid (Table 6.13). These findings suggest that patients with mastalgia are definitely suffering from EFA deficiency, and this is partially correctable by a short course of EPO treatment. The metabolic effects of long term therapy are currently unknown, but a long term study of patients with breast cysts is underway in Cardiff, and this trial has an EPO treatment arm lasting 1 year, which will show whether the EFA deficiencies demonstrated in the mastalgia patients are totally correctable by EPO treatment.

In summary, 4 months treatment with EPO produces a significant, if modest, improvement in cyclical mastalgia symptoms, and partially corrects the demonstrated EFA deficiencies in these patients.

CHAPTER SEVEN

CONCLUSIONS

When the studies described in this thesis were commenced, there was little guidance in the literature on the value or otherwise of drug therapy in the treatment of mastalgia. No systematic work on the problem had been reported since the studies of Atkins were published in the 1940-50 decade. The 3 major trials described in this thesis have enabled a policy of treatment to be formulated. Most patients with mastalgia can be reassured, but about 15% require some therapy. The trials have shown that bromocriptine and danazol are both effective agents for cyclical mastalgia, but with different side effect profiles. Danazol appears to be the most effective drug if absolute overall response rates are calculated (Pye et al 1985, Hinton et al 1986a,Griffith et al 1987). Bromocriptine has a greater number of side effects than danazol, but the latter's tendency to produce amenorrhoea is also a major problem, as many women do not like the idea of drug induced amenorrhoea. A low dose danazol regimen currently under study in Cardiff, appears to substantially reduce the incidence of amenorrhoea and side effects.

There have been few comparative studies of the therapies described in this thesis, but 3 studies have been published, two comparing danazol with bromocriptine and one comparing danazol with tamoxifen. Montgomery et al used a sequential design in 20 patients who were given bromocriptine 2.5mgs b.d. for 3 months, followed by danazol 400mgs daily for 3 months (Montgomery et al 1979). They showed no difference in response rates between the drugs, although no statistical comparisons were made. They reported that the patients found the side effects of danazol more distressing, but no data are given. The second study comparing danazol with tamoxifen randomised 50 patients equally to either danazol 100mgs b.d. or tamoxifen 20mgs/day for 6 months, with no crossover (Powles et al 1987). The response rates of cyclical pain were similar in the 2 groups, 88% for tamoxifen and 80% for danazol, while the patients on danazol had more side effects, but a similar proportion in each group stopped therapy due to side effects. The data of response from the danazol arm of the Powles et al study, agree closely with the data from the double blind study reported in this thesis, but the overall side effects profile was higher in the former study, although the incidence of amenorrhoea was lower. The conclusion of Powles et al that tamoxifen may be a better agent because of lower toxicity, has been overtaken by events as new toxicity studies by ICI have shown liver tumours in rats treated by high dose tamoxifen. A double blind study has recently confirmed the efficacy of tamoxifen in

cyclical mastalgia (Fentiman et al 1986). Fentiman and Powles have recently argued that tamoxifen should still be considered for the treatment of mastalgia despite the adverse rat toxicology data, as it has lower side effects than the other agents and is highly effective (Fentiman and Powles 1987), but the drug is known to have a higher incidence of side effects when used as a prophylactic agent for high risk benign breast disease (J.C. Gazet 1988, personal communication). In any event, tamoxifen is not currently licensed for use in benign conditions, and thus cannot be recommended for general use in mastalgia patients.

The third comparative study reported 47 patients with severe cyclical mastalgia who were allocated bromocriptine, danazol or placebo in a randomised double blind parallel-block design (Hinton et al 1986a). These authors concluded that danazol was significantly better than bromocriptine for reduction of breast pain, heaviness, tenderness and effect on sex life. However, only 34 patients eventually received treatment and the linear analogue data were analysed by parametric statistics, so the result may not be as clear-cut as presented.

In this thesis evening primrose oil was also shown to be significantly better than placebo, although the overall response rate seems to be lower than that for bromocriptine and danazol. However, the agent has very few side effects,does not interfere with menstruation and can be given to patients with milder symptoms. The attractions of a 'natural' product also give a high

compliance rate.

In terms of expense, bromocriptine is the cheapest agent costing only £11/month, compared with danazol £25/month (200 mgs) or £15/month (100 mgs). Evening primrose oil is not currently available on prescription but retails for about £6 for 50 capsules, which would cost about £23/month at the recommended dose for mastalgia. Thus no 'ideal' agent has yet emerged, but the availability of 3 agents allows a change to an alternative therapy if one is ineffective, and so most patients with mastalgia can be helped by one or other of the agents. These trials have shown that about 60-70% of mastalgia patients can be helped by drug therapy, and recent follow up studies have shown that failure of response to one of the agents, does not necessarily predict failure on another (Pye et al 1985)

These studies have also established the value of VLA scales and pain charts in the assessment of breast pain, and represent the first series of detailed studies using these forms of assessment in a systematic way. As a result of publications emanating from these studies, these techniques of assessment have become the norm for mastalgia studies (Watt-Boolsen et al 1985, Durning and Sellwood 1982) and are currently being used in 2 major international trials of mastalgia (Parlodel European Multicentre study and Zoladex International study). The pain charts used in both of these international studies were personally designed by the author and have been translated into the respective languages

of the participating centres. The pain chart and the VLA forms are particularly useful as they are self assessment methods, and can be returned to the investigator by post, so making multiple return visits to the clinic unnecessary. A further novel finding is the apparent lack of placebo effect on the pain charts, found in both the danazol and evening primrose oil studies. This suggests that all future controlled trials in mastalgia should use a pain chart in the assessment, as it may produce a more objective measurement of response to treatment.

These studies have also examined hormonal parameters in mastalgia patients on treatment. Prior to these studies, several investigations had been made of basal hormone levels in benign breast disease (see reviews by Golinger 1978 and Wang and Fentiman 1985), but no consistent abnormalities had been shown despite the promise of accurate measurement of hormones by radio-immunoassay. In conjunction with the therapeutic trials described in this thesis, detailed hormonal assessments have been made, especially of prolactin dynamics. These have led to further work which has demonstrated the value of dynamic hormonal assessment in mastalgia patients (Kumar et al 1984a+b, 1985). These endocrine findings have now been confirmed by several studies in different countries, and have opened up many new avenues of research into the hormonal aetiology of benign breast disease (Dogliotti et al 1985, Peters et al 1981, Watt Boolsen et al 1985). Testing of dynamic prolactin responses to TRH have now become routine in the Cardiff Mastalgia Clinic, and are also being

evaluated in a large European multi-centre study of mastalgia. The evening primrose oil trial has led to new work examining the bioactivity of prolactin and its relationship to dietary essential fatty acid intake (Love and Rose 1985) while currently collaborative work in this exciting area is being carried out in Cardiff, Manchester and the U.S.A.

The publications resulting from the studies reported here have increased the general awareness of mastalgia, especially the cyclical and non-cyclical patterns. As a result authors in Europe and the USA use the terminology developed in Cardiff (Montgomery et al 1979, Durning and Sellwood 1982, London et al 1982, Watt-Boolsen 1983, Herrmann and Walther 1983, Ayers and Gidwani 1983, Walsh et al 1984a+b, Rose et al 1987, Doberl et al 1984, Blichert-Toft et al 1979, Watt-Boolsen et al 1985, Hinton et al 1986a, Leinster et al 1987, Powles et al 1987, Dowle 1987), and it is clear that these studies have influenced thought and work in the field of painful benign breast disease. The paper by Ayers and Gidwani is particularly interesting as it clearly shows that although an increased response of prolactin secretion to TRH occurs in mastalgia patients, it does not correlate with the structural changes of so called "fibrocystic disease" as measured by ultrasonography. Further, Bischoff and Wenderlein have show that there is no correlation between mammographic appearance and patterns of mastalgia (Bischoff and Wenderlein 1979). Watt-Boolsen et al have shown that mastalgia does not correlate with any histological component of fibro-cystic disease (Watt-Boolsen et al

1982). Evidence such as this clearly supports the concept advanced in this thesis, of adopting a symptomatic rather than a pathological approach to mastalgia as suggested 35 years ago by David Patey (Patey 1951). The adoption of the Cardiff terminology, trial methodology and documentation by these different investigators, demonstrates that not only have these studies set the standard, but have also generated much new work and ideas, as good research should.

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APPENDIX I EXAMPLE OF WILCOXON'S SIGNED RANKS TEST

APPENDIX 1 THE WILCOXON MATCHED PAIRS SIGNED RANK TEST

This non-parametric test is distribution-free and is ideal for ordinal data such as that obtained from the VLA scale and simple pain scale. This worked example is taken from the bromocriptine trial VLA data for breast pain in the cyclical patients and compares the individual pretrial scores with those at the end of the first treatment period, ie the placebo score in the placebo -> bromocriptine group and the bromocriptine score in the bromocriptine -> placebo group (Appendix III).

Example (a) The bromocriptine -> placebo group.

(i) Take breast pain VLA scores for the pretrial value and the end of bromocriptine value for each of the patients randomised to bromocriptine as the first treatment (from Appendix III).

	VLA Score	
Trial No.	Pretrial	End of bromocriptine ie cycle 2
3C	94	3
12C	76	4
16C	99	27
19C	76	3
20C	43	8
21C	71	68
26C	66	5
28C	74	11
31C	63	76
32C	45	81
33C	94	85
36C	6	22

(ii) Subtract the bromocriptine score from the pretrial score to find the difference (d) between the scores.

Trial No.	Pretrial	End bromocriptine	Difference(d)
3C	94	3	91
12C	76	4	72
16C	99	27	72
19C	76	3	73
20C	43	8	35
21C	71	68	3
26C	66	5	61
28C	74	11	63
31C	63	76	-13
32C	45	81	-36
33C	94	85	9
36C	6	22	-16

(iii) Rank the differences in order of magnitude starting with the least difference and finishing with the largest. Note the negative differences retain their negative sign.

Trial No.	Pretrial	End bromocriptine	Difference	Rank
3C	94	3	91	3
12C	76	4	72	9
16C	99	27	72	-13
19C	76	3	73	-16
20C	43	8	35	35
21C	71	68	3	-36
26C	66	5	61	61
28C	74	11	63	63
31C	63	76	-13	72
32C	45	81	-36	72
33C	94	85	9	73
36C	6	22	-16	91

(iv) Give each rank a score (positive or negative) starting from the first rank to the last (12th). Equal differences share the ranks and are given 1/2 the score of 2 ranks or 1/3 score of 3 ranks etc.

Trial No.	Pretrial	End Bromo.	Difference	Rank	Rank Score
3C	94	3	91	3	1
12C	76	4	72	9	2
16C	99	27	72	-13	-3
19C	76	3	73	-16	-4
20C	43	8	35	35	5
21C	71	68	3	-36	-6
26C	66	5	61	61	7
28C	74	11	63	63	8
31C	63	76	-13	72	9.5
32C	45	81	-36	72	9.5
33C	94	85	9	73	11
36C	6	22	-16	91	12

(v) Add up the sum of the negative ranks and the positive ranks.
$-3 + -4 + -6 = -131$
$+ 2 + 5 + 7 + 8 + 9.5 + 9.5 + 11 + 12 = +65$
This gives the data shown in line 1 of table 3.4

The significance of the results is looked up in a table of Wilcoxon values for paired observations for the appropriate number of pairs (12 in this case) and the tables show that any distribution of scores equal or less than 13, 65 gives a p value of 0.05 (Short Textbook of Medical Statistics, Hill 1977).

Example (b) The placebo -> bromocriptine group - Breast pain.

This example compares the changes of VLA scores from pretrial to end of placebo in the group randomised to take placebo as the first therapy. The scores are written down as the pretrial and end of placebo (cycle 2).

Trial No	Pretrial	Placebo (Cycle 2)	Difference	Order of Difference	Rank
1C	0	9	-9	-6	-1
7C	14	0	14	-7	-2
8C	70	26	44	8	3
10C	70	95	-25	-9	-4
11C	43	49	-6	14	5
15C	26	33	-7	-25	-6
17C	99	99	0	-27	-7
18C	37	64	-27	-38	-8.5
22C	59	59	0	38	8.5
24C	75	29	46	44	10
25C	28	20	8	46	11
27C	96	9	87	-53	-12
29C	100	100	0	-78	-13
30C	0	78	-78	87	14
34C	73	35	38		
35C	0	53	-53		
37C	38	76	-38		

The rank sums are - 53.5 + 51.5.
These are almost equal and thus the magnitude of negative and positive changes in the patients are about equal and so there appears to be no significant effect of placebo on breast pain. This is confirmed by reference to a Wilcoxon's table which shows that for 14 pairs, a distribution of 21, 84 is needed to achieve a p value of 0.05.(Note that although the calculation began with 17 pairs, 3 pairs showed no difference between the 2 scores and so are dropped from the calculations).The final rank sums of - 53.5 + 51.5 appear on the first line of table 3.5.

APPENDIX II VISUAL LINEAR ANALOGUE PROFORMA

TRIAL No:

NAME OR ADDRESSOGRAPH:

DATE:

SELF-EVALUATION QUESTIONNAIRE (MASTALGIA)

We are trying to find out how people are affected by illness, in the hope that we can improve our methods of treatment. We would, therefore, be pleased if you would complete this questionnaire.

To help you answer, each question is followed by a line, as the following example shows:-

QUESTION: Have you been feeling tired ?



You are asked to mark each line in a way that best indicates how you have been during the period of time under question.

If you had been feeling very tired all the time you would put a mark as follows:



If you had been somewhat tired all the time you would mark the line thus:



PLEASE MAKE SURE THAT YOU COMPLETE EVERY QUESTION. IF A QUESTION DOES NOT APPLY TO YOU PLEASE RING THE WORDS "NOT APPLICABLE".

SECTION I - BREAST PAIN

Have you had any of the following symptoms or problems over the last .....days or.....weeks?

(a) Breast Pain



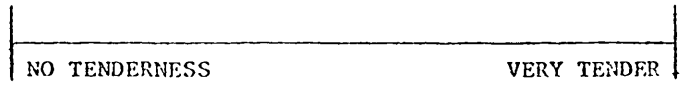
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(b) Breast Heaviness



.....

(c) Tenderness to Touch (breast)



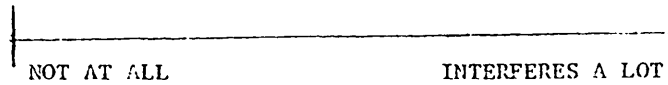
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(d) Sore feeling in the breast



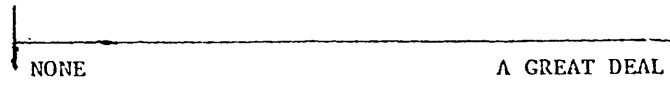
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(e) Does your breast pain affect your sex life?



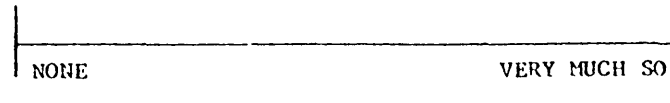
NOT APPLICABLE .....

(f) Nipple Discharge



.....

(g) Any other problem? Specify.....
.....
.....
.....



.....

SECTION 2 - GENERAL HEALTH

Have you had any of the following symptoms or problems over the last .....days or.....weeks.

(a) Headache



.....

(b) Feeling irritable



.....

(c) Feeling depressed or miserable



.....

(d) Bloated feeling in the stomach



.....

(e) Feeling of well being



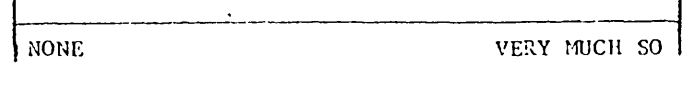
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(f) Feeling tired



.....

(g) Any other problem? Specify.....
.....
.....



.....

SECTION 3 - TREATMENT

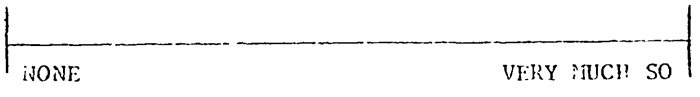
(a) Is treatment helping you?



.....

(b) Any side effects? Please fill in below and mark on the lines.

.....



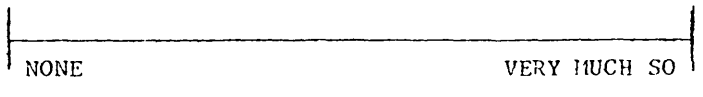
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APPENDIX III RAW DATA BROMOCRIPTINE TRIAL

APPENDIX III BROMOCRIPTINE TRIAL RAW DATA

ENTRY CHARACTERISTICS OF CYCLICAL GROUP

TRIAL NO	AGE (YRS)	CYCLE LENGTH (DAYS)	PAIN DURATION (YRS)
1C	33	27	2
2C	34	31	2
3C	33	26	13
4C	36	26	1.5
5C	29	26	10
6C	31	28	7
7C	27	36	4
8C	33	28	3
9C	37	25	1.5
10C	25	30	4
11C	33	29	1.2
12C	45	25	3
13C	23	21	1
14C	32	28	1
15C	33	*	3
16C	32	26	4
17C	32	*	4
18C	37	*	5
19C	34	31	3
20C	22	27	1.5
21C	36	28	11
22C	38	29	1.5
23C	38	*	3
24C	37	26	6
25C	19	28	1
26C	32	29	20
27C	25	28	2
28C	30	29	2
29C	37	31	10
30C	39	27	23
31C	35	32	3
32C	21	29	1
33C	31	31	5
34C	37	30	5
35C	42	*	5
36C	26	32	1.5
37C	26	28	4
38C	46	*	4

* = Previous hysterectomy

ENTRY CHARACTERISTICS OF THE NON-CYCLICAL GROUP

TRIAL NO	AGE (YRS)	CYCLE LENGTH (DAYS)	PAIN DURATION (YRS)
1S	43	30	0.8
2S	28	27	2.5
3S	51	25	20
4S	37	26	1
5S	NOT ENTERED		NOT ENTERED
6S	58	*	1
7S	43	*	1.5
8S	35	*	5
9S	45	*	1.5
10S	25	28	1
11S	61	*	4
12S	52	14	1
13S	35	28	8
14S	44	*	0.75
15S	36	27	1.5
16S	37	26	1

* = Previous hysterectomy or menopause

PATIENT HV TRIAL NO. 1C CYCLICAL GROUP PLACEBO > BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	74	7	9		21	9	70	54
Breast Heaviness	0	67	9		10	8	-	16
Tenderness to touch	81	67	43		29	50	72	83
Sore feeling	80	5	35		9	50	56	81
Sex life	-	-	-		-	-	-	-
Nipple discharge	1	4	7		5	8	6	7
Headache	85	65	10		12	11	99	66
Feeling irritable	75	91	56		13	18	23	68
Feeling depressed	76	91	12		12	14	12	68
Bloated feeling	0	11	12		11	13	16	13
Well being	-	60	12		10	13	65	78
Feeling tired	95	-	-		-	-	-	-
Treatment helping	N/A	69	78		88	85	29	14
Clinical Assessment								
Tenderness	2	1	1		1	1	2	2
Nodularity	1	1	2		1	1	1	2
Response	N/A	2	1		1	1	2	2
Prolactin mU/100 ml	-	-	-		-	-	-	-

PATIENT J A TRIAL NO. 2 CYCLICAL GROUP BROMOCRIPTINE -> PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	32	0	-		-	-	-	-
Breast Heaviness	17	0	-		-	-	-	-
Tenderness to touch	60	0	-		-	-	-	-
Sore feeling	0	0	-		-	-	-	-
Sex life	0	0	-		-	-	-	-
Nipple discharge	0	0	-		-	-	-	-
Headache	0	0	-		-	-	-	-
Feeling irritable	8	10	-		-	-	-	-
Feeling depressed	4	12	-		-	-	-	-
Bloated feeling	0	0	-		-	-	-	-
Well being	15	11	-		-	-	-	-
Feeling tired	16	0	-		-	-	-	-
Treatment helping	N/A	-	-		-	-	-	-
Clinical Assessment								
Tenderness	2	1	-	-	-	-	-	-
Nodularity	2	2	-	-	-	-	-	-
Response	N/A	2	-	-	-	-	-	-
Prolactin mU/100 ml	-	-	-	-	-	-	-	-

WITHDRAWAL ON CYCLE 1 SIDE EFFECTS

PATIENT VH TRIAL NO. 3C CYCLICAL GROUP BROMOCRIPTINE > PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	94	7	3		82	0	74	100
Breast Heaviness	93	12	5		80	0	75	99
Tenderness to touch	91	19	23		100	2	100	100
Sore feeling	93	16	18		92	6	98	100
Sex life	94	95	3		86	2	68	100
Nipple discharge	0	10	0		0	0	0	0
Headache	98	81	100		98	62	12	74
Feeling irritable	60	86	55		86	44	17	82
Feeling depressed	67	86	65		2	52	21	87
Bloated feeling	100	93	100		45	11	41	77
Well being	52	39	65		21	45	21	70
Feeling tired	92	91	96		-	-	-	-
Treatment helping	N/A	81	91		13	99	6	2
Clinical Assessment								
Tenderness	3	1	1		3	1	2	2
Nodularity	3	1	1		2	1	2	2
Response	N/A	2	1		3	1	2	3
Prolactin mU/100 ml	18	5	14		28	18	20	35

PATIENT SB TRIAL NO. 4 CYCLICAL GROUP PLACEBO -> BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	68	65	31		65	-	-	-
Breast Heaviness	80	82	44		-	-	-	-
Tenderness to touch	100	84	70		92	-	-	-
Sore feeling	81	99	98		79	-	-	-
Sex life	55	55	46		-	-	-	-
Nipple discharge	4	5	3		1	-	-	-
Headache	45	50	62		15	-	-	-
Feeling irritable	5	65	2		32	-	-	-
Feeling depressed	58	50	38		71	-	-	-
Bloated feeling	58	-	98		52	-	-	-
Well being	-	56	84		46	-	-	-
Feeling tired	20	66	70		95	-	-	-
Treatment helping	N/A	-	44		34	-	-	-
Clinical Assessment								
Tenderness	2	2	2		2	-	-	-
Nodularity	1	1	1		2	-	-	-
Response	N/A	3	2		3	-	-	-
Prolactin mU/100 ml	22	11	1		7	-	-	-

WITHDRAWAL IN CYCLE 3 - INTERCURRENT ILLNESS

PATIENT KB TRIAL NO. 5 CYCLICAL GROUP BROMOCRIPTINE -> PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	66	-	-		-	-	-	-
Breast Heaviness	71	-	-		-	-	-	-
Tenderness to touch	74	-	-		-	-	-	-
Sore feeling	75	-	-		-	-	-	-
Sex life	70	-	-		-	-	-	-
Nipple discharge	0	-	-		-	-	-	-
Headache	0	-	-		-	-	-	-
Feeling irritable	70	-	-		-	-	-	-
Feeling depressed	74	-	-		-	-	-	-
Bloated feeling	63	-	-		-	-	-	-
Well being	55	-	-		-	-	-	-
Feeling tired	24	-	-		-	-	-	-
Treatment helping	N/A	-	-		-	-	-	-
Clinical Assessment								
Tenderness	3	2	-		-	-	-	-
Nodularity	3	2	-		-	-	-	-
Response	N/A	2	-		-	-	-	-
Prolactin mU/100 ml			-					

WITHDRAWAL IN CYCLE 1 - SIDE EFFECTS

PATIENT MG TRIAL NO. 6 CYCLICAL GROUP BROMOCRIPTINE -> PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	8	34	11		69	46	32	-
Breast Heaviness	10	10	10		47	15	69	-
Tenderness to touch	85	15	41		83	54	72	-
Sore feeling	56	15	30		86	62	53	-
Sex life	35	33	25		61	49	52	-
Nipple discharge	12	12	15		14	12	-	-
Headache	11	44	43		40	14	48	-
Feeling irritable	28	46	45		46	48	22	-
Feeling depressed	10	12	10		6	4	24	-
Bloated feeling	8	54	10		52	10	53	-
Well being	18	16	20		50	45	44	-
Feeling tired	-	41	-		6	15	46	-
Treatment helping	N/A	46	10		50	12	41	-
Clinical Assessment								
Tenderness	3	1	2		2	1	2	-
Nodularity	3	1	2		2	2	2	-
Response	N/A	2	2		3	2	2	-
Prolactin mU/100 ml	21	2	8		>93	>93	>93	-

WITHDREW IN CYCLE 5 - BECAME PREGNANT

PATIENT AP TRIAL NO. 7C CYCLICAL GROUP PLACEBO > BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	49	74	50		90	80	66	21
Breast Heaviness	14	57	0		75	81	13	0
Tenderness to touch	67	100	74		89	81	52	26
Sore feeling	72	100	75		91	84	13	25
Sex life	79	-	-		-	-	-	-
Nipple discharge	0	0	0		-	0	0	0
Headache	100	100	100		82	17	11	0
Feeling irritable	100	50	19		83	77	14	0
Feeling depressed	50	88	30		85	74	12	0
Bloated feeling	16	80	70		87	76	13	0
Well being	78	0	27		77	71	25	0
Feeling tired	47	14	17		87	100	13	0
Treatment helping	N/A	50	69		13	0	74	90
Clinical Assessment								
Tenderness	2	3	2		2	2	-	2
Nodularity	2	2	2		2	2	-	2
Response	N/A	3	2		3	3	-	2
Prolactin mU/100 ml	10	23	27		7	14	-	4

PATIENT PT TRIAL NO. 8C CYCLICAL GROUP PLACEBO > BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	56	37	30		73	17	45	24
Breast Heaviness	70	20	26		73	20	70	82
Tenderness to touch	64	33	30		73	33	72	54
Sore feeling	63	28	27		74	32	77	32
Sex life	57	40	27		62	27	34	28
Nipple discharge	55	67	66		70	40	74	81
Headache	62	65	66		80	36	55	18
Feeling irritable	66	67	55		79	34	53	28
Feeling depressed	69	67	41		82	38	64	31
Bloated feeling	71	69	20		63	24	72	80
Well being	79	75	50		78	27	53	18
Feeling tired	-	70	50		69	22	70	20
Treatment helping	N/A	80	92		80	95	89	98
Clinical Assessment								
Tenderness	2	1	1		2	1	1	1
Nodularity	3	2	2		3	2	2	2
Response	N/A	1	1		2	1	1	2
Prolactin mU/100 ml	-	-	-		-	-	-	-

PATIENT MD TRIAL NO. 9 (CYCLICAL) GROUP BROMCRIPTINE -> PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	21	0	0		68	-	-	-
Breast Heaviness	77	18	80		71	-	-	-
Tenderness to touch	96	16	77		94	-	-	-
Sore feeling	3	0	0		72	-	-	-
Sex life	100	22	80		73	-	-	-
Nipple discharge	0	0	0		0	-	-	-
Headache	17	8	63		0	-	-	-
Feeling irritable	15	50	0		0	-	-	-
Feeling depressed	3	47	0		0	-	-	-
Bloated feeling	67	26	25		0	-	-	-
Well being	48	53	15		13	-	-	-
Feeling tired	44	-	-		-	-	-	-
Treatment helping	N/A	90	48		39	-	-	-
Clinical Assessment								
Tenderness	3	3	1		2	-	-	-
Nodularity	3	3	2		3	-	-	-
Response	N/A	3	3		2	-	-	-
Prolactin mU/100 ml	16	-	14		7	-	-	-

WITHDRAWAL IN CYCLE 4 - BECAME PREGNANT

PATIENT JC TRIAL NO. 10C CYCLICAL GROUP PLACEBO > BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	71	88	94		18	94	100	48
Breast Heaviness	70	86	95		47	93	100	50
Tenderness to touch	100	98	100		31	98	100	30
Sore feeling	100	99	98		7	100	100	22
Sex life	-	-	-		-	-	-	-
Nipple discharge	0	0	0		0	0	0	0
Headache	100	100	100		100	97	100	93
Feeling irritable	0	54	49		0	32	52	11
Feeling depressed	25	55	53		16	34	77	11
Bloated feeling	36	0	53		0	34	93	13
Well being	65	75	51		96	87	94	82
Feeling tired	46	22	-		-	-	54	6
Treatment helping	N/A	2	3		79	10	0	10
Clinical Assessment								
Tenderness	3	3	2		1	2	2	1
Nodularity	3	3	2		2	2	3	2
Response	N/A	3	2		1	2	3	2
Prolactin mU/100 ml	-	35	40		4	8	17	-

PATIENT JH TRIAL NO. 11C CYCLICAL GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	21	24	10		1	1	13	17
Breast Heaviness	43	26	49		8	23	30	47
Tenderness to touch	1	1	9		2	0	3	15
Sore feeling	41	1	4		4	1	3	6
Sex life	0	0	0		0	0	0	0
Nipple discharge	16	6	34		0	1	1	2
Headache	60	0	0		0	0	4	0
Feeling irritable	82	1	9		5	10	2	0
Feeling depressed	32	1	18		9	4	51	5
Bloated feeling	76	30	43		32	30	68	43
Well being	47	1	1		9	1	2	4
Feeling tired	91	-	-		-	0	2	14
Treatment helping	N/A	10	5		43	33	68	69
Clinical Assessment								
Tenderness	2	1	2		1	1	1	1
Nodularity	1	1	1		2	1	1	1
Response	N/A	2	3		2	2	2	1
Prolactin mU/100 ml	20	4	-		-	20	9	17

PATIENT MD TRIAL NO. 12C CYCLICAL GROUP BROMOCRIPTINE → PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	76	12	4		1	1	17	1
Breast Heaviness	92	18	3		0	2	2	1
Tenderness to touch	93	30	4		0	1	2	75
Sore feeling	94	29	4		0	0	2	3
Sex life	69	40	-		-	-	-	-
Nipple discharge	37	0	3		0	0	1	2
Headache	95	70	85		12	18	68	67
Feeling irritable	95	21	35		13	30	28	67
Feeling depressed	95	24	35		1	2	3	1
Bloated feeling	95	10	2		0	19	3	72
Well being	59	51	22		20	78	22	76
Feeling tired	90	-	-		75	25	100	99
Treatment helping	N/A	56	100		100	96	100	100
Clinical Assessment								
Tenderness	3	2	1		1	1	1	1
Nodularity	2	1	1		1	1	2	1
Response	N/A	2	1		1	1	2	1
Prolactin mU/100 ml	56	24	69		51	56	55	54

PATIENT PC TRIAL NO. 13 CYCLICAL GROUP BROMOCRIPTINE -> PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	72	75	-		-	-	-	-
Breast Heaviness	81	60	-		-	-	-	-
Tenderness to touch	99	85	-		-	-	-	-
Sore feeling	94	64	-		-	-	-	-
Sex life	0	0	-		-	-	-	-
Nipple discharge	0	0	-		-	-	-	-
Headache	15	9	-		-	-	-	-
Feeling irritable	0	44	-		-	-	-	-
Feeling depressed	45	24	-		-	-	-	-
Bloated feeling	0	41	-		-	-	-	-
Well being	71	29	-		-	-	-	-
Feeling tired	13	0	-		-	-	-	-
Treatment helping	N/A	10	-		-	-	-	-
Clinical Assessment								
Tenderness	3	3	-		-	-	-	-
Nodularity	3	3	-		-	-	-	-
Response	N/A	3	-		-	-	-	-
Prolactin mU/100 ml		<6	-		-	-	-	-

WITHDRAWAL IN CYCLE 2 - DNA'd

PATIENT CR TRIAL NO. 14 CYCLICAL GROUP PLACEBO -> BROMOCRIPTINE DOSE 2.5mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	48	-	-		-	-	-	-
Breast Heaviness	73	-	-		-	-	-	-
Tenderness to touch	72	-	-		-	-	-	-
Sore feeling	76	-	-		-	-	-	-
Sex life	35	-	-		-	-	-	-
Nipple discharge	0	-	-		-	-	-	-
Headache	68	-	-		-	-	-	-
Feeling irritable	60	-	-		-	-	-	-
Feeling depressed	48	-	-		-	-	-	-
Bloated feeling	86	-	-		-	-	-	-
Well being	54	-	-		-	-	-	-
Feeling tired	99	-	-		-	-	-	-
Treatment helping	N/A	-	-		-	-	-	-
Clinical Assessment								
Tenderness	3	-	-		-	-	-	-
Nodularity	2	-	-		-	-	-	-
Response	N/A	-	-		-	-	-	-
Prolactin mU/100 ml	20	-	-		-	-	-	-

WITHDRAWAL IN CYCLE 1 - DNA'd

PATIENT PS TRIAL NO. 15C CYCLICAL GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	39	52	54		62	47	30	59
Breast Heaviness	26	11	33		29	27	20	35
Tenderness to touch	50	15	65		57	73	36	54
Sore feeling	43	46	44		63	47	40	64
Sex life	36	24	24		30	16	30	16
Nipple discharge	0	0	0		0	0	0	1
Headache	50	53	48		72	57	54	59
Feeling irritable	45	56	40		68	66	45	49
Feeling depressed	34	47	31		64	54	51	55
Bloated feeling	0	1	1		8	6	18	1
Well being	36	40	41		59	56	50	60
Feeling tired	33	60	70		80	71	66	69
Treatment helping	N/A	39	24		32	46	39	49
Clinical Assessment								
Tenderness	2	2	2		2	2	2	2
Nodularity	2	2	2		2	1	1	2
Response	N/A	2	3		2	3	3	2
Prolactin mU/100 ml	-	-	-		-	-	-	-

PATIENT BS TRIAL NO. 16C CYCLICAL GROUP BROMOCRIPTINE → PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	99	99	27		94	85	78	80
Breast Heaviness	55	88	19		94	68	78	85
Tenderness to touch	100	100	36		90	95	45	95
Sore feeling	100	98	23		99	95	88	87
Sex life	70	59	-		-	-	-	-
Nipple discharge	1	0	0		0	0	0	0
Headache	99	50	0		60	8	49	12
Feeling irritable	89	73	20		91	28	65	19
Feeling depressed	99	89	16		100	25	56	18
Bloated feeling	58	0	0		0	-	0	0
Well being	100	72	38		86	29	49	26
Feeling tired	-	86	-		85	64	85	21
Treatment helping	N/A	1	23		12	19	55	12
Clinical Assessment								
Tenderness	2	2	2		2	2	2	2
Nodularity	1	1	1		2	2	2	2
Response	N/A	3	3		2	3	3	3
Prolactin mU/100 ml	7	18	18		90	65	27	34

PATIENT CD TRIAL NO. 17C CYCLICAL GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	98	35	98		2	2	2	1
Breast Heaviness	99	36	99		2	2	1	1
Tenderness to touch	98	34	98		2	2	1	1
Sore feeling	99	29	97		12	2	1	1
Sex life	100	1	52		1	2	1	1
Nipple discharge	1	1	1		1	2	1	1
Headache	99	17	42		2	2	2	1
Feeling irritable	59	1	80		2	2	2	1
Feeling depressed	43	2	4		2	1	2	1
Bloated feeling	2	2	3		2	1	2	1
Well being	35	4	49		3	1	2	1
Feeling tired	97	1	50		2	2	1	1
Treatment helping	N/A	92	25		75	99	98	99
Clinical Assessment								
Tenderness	2	1	2		1	1	1	1
Nodularity	2	2	2		1	1	1	2
Response	N/A	2	3		1	1	1	1
Prolactin mU/100 ml	10	23	40		13	23	-	65

PATIENT DH TRIAL NO. 18C CYCLICAL GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	42	35	60		20	-	17	75
Breast Heaviness	37	24	64		-	-	17	100
Tenderness to touch	50	100	72		42	100	75	100
Sore feeling	43	28	-		100	22	44	100
Sex life	9	38	55		16	0	0	60
Nipple discharge	0	0	0		0	0	0	14
Headache	0	100	0		0	0	0	56
Feeling irritable	62	100	69		16	40	45	100
Feeling depressed	60	100	76		19	39	0	100
Bloated feeling	100	100	38		100	54	16	100
Well being	80	100	61		36	16	22	100
Feeling tired	100	100	63		29	69	39	100
Treatment helping	N/A	70	17		22	47	0	26
Clinical Assessment								
Tenderness	2	2	2		1	-	1	2
Nodularity	2	2	2		2	-	2	2
Response	N/A	3	3		3	-	2	3
Prolactin mU/100 ml	16	17	9		1	-	10	25

PATIENT SW TRIAL NO. 19C CYCLICAL GROUP BROMOCRIPTINE → PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	76	1	3		92	75	77	71
Breast Heaviness	77	13	3		92	74	74	74
Tenderness to touch	75	1	12		91	72	72	70
Sore feeling	72	0	12		92	72	73	70
Sex life	99	3	11		91	68	70	64
Nipple discharge	3	1	1		3	0	6	4
Headache	63	27	52		45	69	69	6
Feeling irritable	27	0	2		8	6	47	7
Feeling depressed	28	0	15		21	46	48	6
Bloated feeling	30	1	50		44	68	72	50
Well being	54	2	52		48	51	47	9
Feeling tired	53	3	19		46	51	74	11
Treatment helping	N/A	99	99		5	44	4	48
Clinical Assessment								
Tenderness	2	1	1		3	-	2	2
Nodularity	2	1	1		2	-	2	2
Response	N/A	1	1		2	-	3	2
Prolactin mU/100 ml	15	10	15		26	17	23	23

PATIENT MP TRIAL NO. 20C CYCLICAL GROUP BROMOCRIPTINE > PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	43	10	8		50	64	35	15
Breast Heaviness	99	0	0		100	72	100	20
Tenderness to touch	100	0	0		100	100	100	21
Sore feeling	100	1	0		52	100	99	15
Sex life	43	0	1		61	61	0	6
Nipple discharge	1	0	0		1	0	15	2
Headache	46	23	10		63	73	53	100
Feeling irritable	100	9	5		97	100	0	65
Feeling depressed	100	1	0		61	100	79	22
Bloated feeling	40	0	0		1	1	1	1
Well being	100	13	11		100	100	55	59
Feeling tired	45	75	21		100	100	61	65
Treatment helping	N/A	100	100		31	16	24	33
Clinical Assessment								
Tenderness	3	1	1		2	2	2	1
Nodularity	3	2	1		3	3	3	1
Response	N/A	3	1		3	3	2	1
Prolactin mU/100 ml	32	18	14		48	98	48	65

PATIENT CS TRIAL NO. 21C CYCLICAL GROUP BROMOCRIPTINE → PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	71	26	68		79	86	27	10
Breast Heaviness	100	0	17		100	92	21	4
Tenderness to touch	100	18	75		100	91	23	3
Sore feeling	0	0	0		100	90	23	0
Sex life	14	0	7		68	25	0	3
Nipple discharge	0	0	0		0	0	0	0
Headache	0	32	7		8	0	0	0
Feeling irritable	100	100	100		100	100	28	14
Feeling depressed	6	0	0		62	80	8	0
Bloated feeling	11	0	12		60	77	6	3
Well being	0	48	48		65	73	47	44
Feeling tired	0	41	77		83	65	0	0
Treatment helping	N/A	29	8		0	0	9	9
Clinical Assessment								
Tenderness	2	1	1		3	2	1	2
Nodularity	2	2	1		2	2	1	2
Response	N/A	2	1		3	3	1	2
Prolactin mU/100 ml	25	12	27		44	20	27	22

PATIENT JB TRIAL NO. 22C CYCLICAL GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	70	65	74		52	53	61	74
Breast Heaviness	59	65	59		61	60	27	60
Tenderness to touch	82	59	65		45	72	39	52
Sore feeling	27	31	32		30	35	26	37
Sex life	15	77	85		30	68	13	25
Nipple discharge	7	6	12		6	4	10	4
Headache	85	71	47		42	52	52	51
Feeling irritable	98	84	79		76	81	66	67
Feeling depressed	65	64	80		64	74	63	69
Bloated feeling	15	22	50		28	30	30	33
Well being	23	59	79		53	58	45	61
Feeling tired	91	91	85		76	76	64	71
Treatment helping	N/A	17	12		23	34	36	34
Clinical Assessment								
Tenderness	2	2	2		2	-	2	2
Nodularity	2	2	2		2	-	2	2
Response	N/A	3	3		3	-	2	2
Prolactin mU/100 ml	15	37.5	25		18	-	26	25

PATIENT ND TRIAL NO. 23 CYCLICAL GROUP BROMOCRIPTINE --> PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	10	9	-		-	-	-	-
Breast Heaviness	15	5	-		-	-	-	-
Tenderness to touch	14	0	-		-	-	-	-
Sore feeling	46	10	-		-	-	-	-
Sex life	33	9	-		-	-	-	-
Nipple discharge	0	0	-		-	-	-	-
Headache	70	85	-		-	-	-	-
Feeling irritable	68	46	-		-	-	-	-
Feeling depressed	64	35	-		-	-	-	-
Bloated feeling	55	14	-		-	-	-	-
Well being	81	88	-		-	-	-	-
Feeling tired	74	91	-		-	-	-	-
Treatment helping	N/A	64	-		-	-	-	-
Clinical Assessment								
Tenderness	2	2	-		-	-	-	-
Nodularity	2	2	-		-	-	-	-
Response	N/A	2	-		-	-	-	-
Prolactin mU/100 ml			-		-	-	-	-

WITHDRAWAL IN CYCLE 2 - CHOLECYSTITIS

PATIENT BM TRIAL NO.24C CYCLICAL GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	73	74	73		4	71	0	92
Breast Heaviness	75	75	29		4	37	0	100
Tenderness to touch	100	95	67		7	100	0	100
Sore feeling	100	74	81		4	90	0	94
Sex life	100	100	78		-	-	0	-
Nipple discharge	0	0	0		0	0	0	0
Headache	66	0	0		98	50	0	84
Feeling irritable	100	0	64		83	0	0	100
Feeling depressed	57	14	60		100	0	0	63
Bloated feeling	71	0	32		84	87	0	100
Well being	60	0	17		91	15	0	78
Feeling tired	48	28	81		100	38	52	100
Treatment helping	N/A	0	31		100	29	100	0
Clinical Assessment								
Tenderness	2	3	3		1	2	1	2
Nodularity	2	3	3		2	2	2	2
Response	N/A	3	3		2	2	1	2
Prolactin mU/100 ml	18	18	13		1	5	14	15

PATIENT AE TRIAL NO. 25C CYCLICAL GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	36	11	62		6	0	14	12
Breast Heaviness	28	37	20		10	14	8	39
Tenderness to touch	57	100	81		25	54	64	90
Sore feeling	0	29	20		0	0	6	8
Sex life	-	-	-		-	-	-	-
Nipple discharge	0	0	0		0	0	0	0
Headache	0	0	0		29	0	13	0
Feeling irritable	22	0	0		0	0	0	0
Feeling depressed	23	0	0		0	0	0	0
Bloated feeling	0	0	22		20	65	20	34
Well being	30	0	0		0	40	0	0
Feeling tired	20	0	0		0	0	55	0
Treatment helping	N/A	0	0		38	24	15	18
Clinical Assessment								
Tenderness	2	3	3		2	2	2	1
Nodularity	2	3	3		2	2	2	2
Response	N/A	3	3		2	2	3	3
Prolactin mU/100 ml	44	36	7		13	18	14	24

PATIENT MH TRIAL NO. 26C CYCLICAL GROUP BROMOCRIPTINE → PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	66	10	5		96	90	86	72
Breast Heaviness	60	8	6		97	88	79	84
Tenderness to touch	60	12	5		99	83	79	87
Sore feeling	41	7	5		98	82	54	84
Sex life	76	9	3		100	86	87	86
Nipple discharge	10	7	2		3	6	5	6
Headache	11	86	12		58	9	17	19
Feeling irritable	77	38	18		98	88	91	50
Feeling depressed	19	4	3		99	10	30	9
Bloated feeling	55	5	4		97	45	45	53
Well being	44	37	3		98	57	61	51
Feeling tired	36	12	2		97	45	91	50
Treatment helping	N/A	90	98		3	6	4	4
Clinical Assessment								
Tenderness	3	1	1		2	3	3	3
Nodularity	3	2	2		2	2	3	3
Response	N/A	1	1		3	3	2	2
Prolactin mU/100 ml	17	3	15		11	14	13	20

PATIENT JS TRIAL NO. 27C CYCLICAL GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	92	46	9		0	0	47	0
Breast Heaviness	96	45	9		30	0	48	65
Tenderness to touch	97	47	11		8	46	39	0
Sore feeling	99	15	11		10	47	99	0
Sex life	51	49	13		0	0	19	0
Nipple discharge	3	2	0		0	0	0	0
Headache	0	1	80		0	47	70	100
Feeling irritable	100	74	80		30	68	73	26
Feeling depressed	99	97	49		46	0	54	56
Bloated feeling	0	2	99		70	0	0	0
Well being	55	19	82		50	49	42	54
Feeling tired	99	74	48		53	73	71	87
Treatment helping	N/A	36	90		99	100	100	100
Clinical Assessment								
Tenderness	3	1	1		1	1	1	1
Nodularity	3	2	2		1	1	1	1
Response	N/A	2	2		2	3	2	1
Prolactin mU/100 ml	38	31	30		34	2	2	3

PATIENT AJ TRIAL NO. 28C CYCLICAL GROUP BROMOCRIPTINE → PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	74	38	11		75	100	50	15
Breast Heaviness	50	24	9		38	45	37	0
Tenderness to touch	28	16	0		44	72	28	0
Sore feeling	21	18	0		32	78	29	5
Sex life	-	-	-		-	-	-	-
Nipple discharge	0	0	0		0	0	0	0
Headache	29	41	60		100	11	27	0
Feeling irritable	12	13	0		32	12	9	0
Feeling depressed	0	19	0		15	6	10	0
Bloated feeling	42	27	23		42	51	45	0
Well being	76	49	43		82	77	52	9
Feeling tired	77	50	41		81	73	55	0
Treatment helping	N/A	21	51		34	0	45	83
Clinical Assessment								
Tenderness	3	1	1		1	2	1	1
Nodularity	3	2	2		2	2	2	2
Response	N/A	2	2		3	3	2	2
Prolactin mU/100 ml	31	16	19		24	-	12	16

PATIENT BH TRIAL NO. 29C CYCLICAL GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	-	41	0		0	66	27	54
Breast Heaviness	100	47	100		0	70	55	0
Tenderness to touch	100	75	100		0	78	70	49
Sore feeling	75	75	38		0	54	51	60
Sex life	0	0	0		0	0	0	0
Nipple discharge	0	0	0		0	0	0	0
Headache	0	0	44		0	28	0	0
Feeling irritable	100	21	0		0	33	0	0
Feeling depressed	65	20	0		0	32	0	0
Bloated feeling	45	36	46		0	40	0	0
Well being	-	42	0		0	41	0	0
Feeling tired	50	40	100		0	89	46	24
Treatment helping	N/A	59	19		100	51	48	0
Clinical Assessment								
Tenderness								
Nodularity								
Response	N/A							
Prolactin mU/100 ml	23	35	17		-	2	5	4

PATIENT LM TRIAL NO. 30C CYCLICAL GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	100	80	72		89	77	67	60
Breast Heaviness	0	0	78		88	78	66	58
Tenderness to touch	100	82	75		82	79	72	62
Sore feeling	100	78	75		82	79	77	63
Sex life	-	-	-		-	-	-	-
Nipple discharge	88	77	75		55	65	66	22
Headache	0	6	-		11	55	46	15
Feeling irritable	77	65	-		69	70	59	32
Feeling depressed	0	0	76		70	76	60	34
Bloated feeling	77	57	77		69	-	37	51
Well being	-	33	80		69	63	49	30
Feeling tired	88	70	80		72	65	62	24
Treatment helping	N/A	74	74		81	45	54	64
Clinical Assessment								
Tenderness	3	2	1		1	3	3	2
Nodularity	3	3	2		2	3	2	2
Response	N/A	2	3		1	3	3	3
Prolactin mU/100 ml	18	22	27		3	2	2	23

PATIENT BMcG TRIAL NO. 31C CYCLICAL GROUP BROMOCRIPTINE → PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	63	13	76		95	10	2	29
Breast Heaviness	58	0	45		66	38	2	31
Tenderness to touch	56	11	78		97	6	8	4
Sore feeling	57	11	78		60	11	2	16
Sex life	74	65	80		100	9	5	31
Nipple discharge	0	0	0		0	0	1	0
Headache	0	98	0		2	0	1	0
Feeling irritable	94	95	65		100	91	1	33
Feeling depressed	92	95	61		100	92	1	25
Bloated feeling	75	0	0		0	0	0	95
Well being	84	85	35		93	79	20	41
Feeling tired	59	99	30		3	50	0	0
Treatment helping	N/A	79	8		0	9	89	0
Clinical Assessment								
Tenderness	3	2	3		2	3	1	3
Nodularity	3	1	2		3	2	1	2
Response	N/A	2	2		3	3	1	3
Prolactin mU/100 ml	32	24	24		-	34	25	26

PATIENT NH TRIAL NO. 32C CYCLICAL GROUP BROMOCRIPTINE → PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	45	90	81		100	2	0	0
Breast Heaviness	24	59	100		100	6	0	0
Tenderness to touch	75	100	100		100	24	0	0
Sore feeling	72	100	89		100	25	0	0
Sex life	9	0	4		44	0	0	0
Nipple discharge	0	0	0		0	0	0	0
Headache	11	12	0		0	0	0	0
Feeling irritable	100	100	4		3	4	2	0
Feeling depressed	15	82	3		0	5	0	0
Bloated feeling	50	44	100		36	0	0	0
Well being	100	91	6		27	27	2	2
Feeling tired	100	100	3		16	25	3	4
Treatment helping	N/A	4	47		0	100	100	100
Clinical Assessment								
Tenderness	2	3	2		3	2	1	1
Nodularity	3	3	3		2	2	1	1
Response	N/A	3	3		3	2	1	1
Prolactin mU/100 ml	41	19	50		23	19	16	-

PATIENT LS TRIAL NO. 33C CYCLICAL GROUP BROMOCRIPTINE → PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	94	83	85		84	82	78	74
Breast Heaviness	85	77	87		82	81	78	77
Tenderness to touch	86	92	93		86	93	94	86
Sore feeling	86	69	92		84	95	91	74
Sex life	67	45	77		-	-	-	-
Nipple discharge	0	0	0		0	0	0	0
Headache	37	18	21		7	38	13	44
Feeling irritable	61	87	66		66	87	47	65
Feeling depressed	60	87	65		64	88	50	66
Bloated feeling	72	67	68		66	57	49	65
Well being	66	72	65		49	77	69	72
Feeling tired	86	85	93		74	74	67	70
Treatment helping	N/A	0	0		0	10	5	0
Clinical Assessment								
Tenderness	3	1	3		3	3	3	2
Nodularity	3	3	3		3	3	3	2
Response	N/A	3	3		3	3	3	3
Prolactin mU/100 ml	33	2	2		31	49	34	8

PATIENT SC TRIAL NO. 34C CYCLICAL GROUP PLACEBO ⟶ BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	19	100	23		21	3	5	0
Breast Heaviness	73	100	35		35	14	0	0
Tenderness to touch	78	100	36		38	17	0	0
Sore feeling	75	62	25		15	5	5	0
Sex life	88	-	29		70	11	2	0
Nipple discharge	0	-	2		0	0	0	0
Headache	0	16	0		14	0	0	0
Feeling irritable	64	41	19		14	4	0	0
Feeling depressed	22	0	11		0	0	0	0
Bloated feeling	49	79	41		0	5	15	0
Well being	37	45	48		19	2	0	0
Feeling tired	9	41	50		10	0	0	0
Treatment helping	N/A	22	34		10	100	100	100
Clinical Assessment								
Tenderness	3	1	1		2	1	1	1
Nodularity	2	2	2		2	2	1	1
Response	N/A	2	2		2	1	1	1
Prolactin mU/100 ml	17	22	6		16	20	13	-

PATIENT OB TRIAL NO. 35C CYCLICAL GROUP PLACEBO ⟶ BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	0	55	58		0	40	0	0
Breast Heaviness	0	54	53		11	48	12	0
Tenderness to touch	0	58	55		0	50	22	0
Sore feeling	0	61	50		0	47	0	0
Sex life	27	52	17		0	0	18	0
Nipple discharge	0	0	0		0	0	0	0
Headache	10	38	69		0	38	44	27
Feeling irritable	30	49	54		11	47	51	37
Feeling depressed	15	23	56		17	70	75	36
Bloated feeling	15	55	51		22	67	83	40
Well being	57	9	59		30	65	62	34
Feeling tired	52	50	55		22	64	50	71
Treatment helping	N/A	64	30		100	66	48	100
Clinical Assessment								
Tenderness	1	2	1		1	1	2	1
Nodularity	2	1	1		1	1	2	1
Response	N/A	2	2		1	2	2	1
Prolactin mU/100 ml	4	15	13		2	2	2	5

PATIENT JS TRIAL NO. 36C CYCLICAL GROUP BROMOCRIPTINE⟶ PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	6	13	22		35	0	0	33
Breast Heaviness	3	0	0		0	0	0	0
Tenderness to touch	9	10	0		28	0	0	17
Sore feeling	5	0	0		0	0	0	0
Sex life	-	0	0		0	0	0	0
Nipple discharge	-	0	0		0	0	0	0
Headache	0	11	0		0	0	0	0
Feeling irritable	0	7	0		0	20	0	0
Feeling depressed	0	0	51		39	0	0	0
Bloated feeling	0	10	47		0	0	0	0
Well being	0	47	60		56	45	0	43
Feeling tired	0	55	100		60	0	0	18
Treatment helping	N/A	100	100		100	100	100	100
Clinical Assessment								
Tenderness	1	1	1		1	1	1	1
Nodularity	3	3	2		2	2	2	2
Response	N/A	1	1		2	3	3	2
Prolactin mU/100 ml	18	2	2		28	36	17	16

PATIENT MJ TRIAL NO. 37C CYCLICAL GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	92	40	94		27	34	39	94
Breast Heaviness	38	49	76		3	1	2	5
Tenderness to touch	91	71	95		42	34	3	3
Sore feeling	80	62	95		39	28	42	11
Sex life	50	-	96		39	97	96	96
Nipple discharge	0	1	2		2	1	0	2
Headache	8	2	1		91	1	2	2
Feeling irritable	95	98	96		50	97	97	75
Feeling depressed	58	62	94		39	45	76	2
Bloated feeling	61	97	2		1	2	56	3
Well being	88	80	45		4	21	2	20
Feeling tired	90	33	78		95	72	95	22
Treatment helping	N/A	3	2		92	95	79	94
Clinical Assessment								
Tenderness	3	1	3		2	1	1	1
Nodularity	2	2	2		1	1	1	1
Response	N/A	3	3		2	2	1	2
Prolactin mU/100 ml	7	2	8		2	6	2	2

PATIENT SB TRIAL NO. 38 (CYCLICAL) GROUP Bromocriptine -> Placebo DOSE 2.5mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	56	-	-		-	-	-	-
Breast Heaviness	45	-	-		-	-	-	-
Tenderness to touch	63	-	-		-	-	-	-
Sore feeling	49	-	-		-	-	-	-
Sex life	100	-	-		-	-	-	-
Nipple discharge	0	-	-		-	-	-	-
Headache	36	-	-		-	-	-	-
Feeling irritable	81	-	-		-	-	-	-
Feeling depressed	62	-	-		-	-	-	-
Bloated feeling	76	-	-		-	-	-	-
Well being	62	-	-		-	-	-	-
Feeling tired	72	-	-		-	-	-	-
Treatment helping	N/A	-	-		-	-	-	-
Clinical Assessment								
Tenderness	3	-	-		-	-	-	-
Nodularity	1	-	-		-	-	-	-
Response	N/A	-	-		-	-	-	-
Prolactin mU/100 ml	12	-	-		-	-	-	-

WITHDRAWAL IN CYCLE 1 - SIDE EFFECTS (Nausea and headache)

PATIENT MH TRIAL NO. 1S (NON-CYCLICAL) GROUP BROMOCRIPTINE → PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	77	24	74		35	62	82	71
Breast Heaviness	18	24	42		29	32	53	42
Tenderness to touch	57	46	79		50	64	84	73
Sore feeling	89	30	68		41	54	80	68
Sex life	49	31	64		33	40	77	63
Nipple discharge	2	3	8		12	3	15	16
Headache	73	27	25		29	18	23	42
Feeling irritable	27	17	9		22	23	57	49
Feeling depressed	3	16	14		11	29	48	68
Bloated feeling	3	3	13		13	18	15	20
Well being	47	48	55		52	56	67	68
Feeling tired	-	-	-		-	-	-	-
Treatment helping	N/A	33	15		64	49	13	11
Clinical Assessment								
Tenderness	2	2	2		2	2	3	-
Nodularity	2	2	1		1	1	2	-
Response	N/A							
Prolactin mU/100 ml	-	-	-		-	-	-	-

PATIENT MR TRIAL NO. 2S (NON-CYCLICAL) GROUP Placebo -> Bromocriptine DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	0	0	0		-	-	-	-
Breast Heaviness	0	43	87		-	-	-	-
Tenderness to touch	22	18	99		-	-	-	-
Sore feeling	20	99	87		-	-	-	-
Sex life	99	44	94		-	-	-	-
Nipple discharge	0	0	0		-	-	-	-
Headache	16	0	98		-	-	-	-
Feeling irritable	44	79	91		-	-	-	-
Feeling depressed	16	78	99		-	-	-	-
Bloated feeling	0	0	0		-	-	-	-
Well being	18	63	92		-	-	-	-
Feeling tired	45	0	0		-	-	-	-
Treatment helping	N/A	0	0		-	-	-	-
Clinical Assessment								
Tenderness	1	1	1		-	-	-	-
Nodularity	1	1	1		-	-	-	-
Response	N/A	3	3		-	-	-	-
Prolactin mU/100 ml	-	-	-		-	-	-	-

WITHDRAWAL IN CYCLE 2 - DNA'd

PATIENT JN TRIAL NO. 3S (NON-CYCLICAL) GROUP BROMOCRIPTINE → PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	61	33	18		68	42	9	36
Breast Heaviness	36	37	17		68	66	14	3
Tenderness to touch	83	38	72		75	65	12	31
Sore feeling	84	48	23		63	45	13	30
Sex life	29	35	20		16	18	18	3
Nipple discharge	4	6	10		10	9	14	2
Headache	54	55	89		89	84	27	68
Feeling irritable	52	8	8		13	14	9	8
Feeling depressed	21	8	7		17	9	9	7
Bloated feeling	61	5	5		8	8	10	5
Well being	33	18	7		59	24	12	39
Feeling tired	-	-	-		-	-	-	-
Treatment helping	N/A	64	75		49	68	80	62
Clinical Assessment								
Tenderness	-	3	2		2	2	2	2
Nodularity	-	1	1		1	1	2	2
Response	N/A	2	1		3	1	1	2
Prolactin mU/100 ml	-	-	-		-	-	-	-

PATIENT JM TRIAL NO. 4S (NON-CYCLICAL) GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	19	25	9		9	9	9	45
Breast Heaviness	43	44	11		11	11	10	34
Tenderness to touch	57	58	41		30	31	30	71
Sore feeling	32	62	25		26	25	33	53
Sex life	67	64	24		24	24	23	22
Nipple discharge	10	11	12		11	10	12	11
Headache	64	68	20		11	10	15	47
Feeling irritable	100	69	28		28	27	31	52
Feeling depressed	32	54	27		28	27	28	28
Bloated feeling	85	73	9		10	9	9	73
Well being	28	46	18		22	18	19	54
Feeling tired	81	60	-		-	-	-	-
Treatment helping	N/A	40	74		73	74	72	73
Clinical Assessment								
Tenderness	2	2	1		1	1	1	2
Nodularity	2	2	1		1	1	1	1
Response	N/A	2	1		1	1	1	2
Prolactin mU/100 ml	16	21	19		8	82	-	20

PATIENT NP TRIAL NO. 6S (NON-CYCLICAL) GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	44	61	55		64	68	58	69
Breast Heaviness	0	61	0		53	53	56	55
Tenderness to touch	100	99	61		99	100	100	100
Sore feeling	46	63	66		63	35	69	54
Sex life	44	0	-		0	-	0	-
Nipple discharge	1	0	0		0	0	0	0
Headache	100	65	100		100	63	52	100
Feeling irritable	40	53	50		41	0	0	0
Feeling depressed	41	60	40		44	41	0	43
Bloated feeling	0	0	43		40	68	52	54
Well being	46	69	45		45	37	46	100
Feeling tired	-	-	-		-	-	56	35
Treatment helping	N/A	1	43		1	0	55	0
Clinical Assessment								
Tenderness	2	2	2		2	2	2	2
Nodularity	1	2	1		1	1	1	1
Response	N/A	3	3		3	3	2	3
Prolactin mU/100 ml	12	13	18		9	4	4	25

PATIENT DS TRIAL NO. 7S (NON-CYCLICAL) GROUP BROMOCRIPTINE → PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	49	3	13		51	29	0	0
Breast Heaviness	24	0	14		22	24	0	0
Tenderness to touch	54	19	49		100	100	0	0
Sore feeling	31	16	4		54	25	0	0
Sex life	96	0	0		-	-	0	0
Nipple discharge	11	0	0		0	0	0	0
Headache	24	100	0		0	49	100	0
Feeling irritable	25	0	0		0	43	0	0
Feeling depressed	0	53	0		0	100	0	0
Bloated feeling	68	0	30		0	44	0	19
Well being	55	100	0		0	100	0	0
Feeling tired	100	-	-		0	100	53	47
Treatment helping	N/A	43	100		78	0	100	100
Clinical Assessment								
Tenderness	2	2	3		2	3	2	1
Nodularity	2	2	2		2	2	2	1
Response	N/A	3	2		2	2	1	1
Prolactin mU/100 ml		15	10		15	33	-	24

PATIENT CB TRIAL NO. 8S (NON-CYCLICAL) GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	65	39	62		48	13	39	15
Breast Heaviness	55	10	31		19	13	34	15
Tenderness to touch	100	69	20		21	13	35	15
Sore feeling	73	66	27		29	11	10	18
Sex life	16	11	0		0	0	0	0
Nipple discharge	80	18	44		10	13	16	15
Headache	83	37	58		73	57	72	44
Feeling irritable	24	26	25		24	39	24	15
Feeling depressed	66	27	13		11	27	12	14
Bloated feeling	14	0	74		81	24	36	8
Well being	20	43	24		9	18	10	4
Feeling tired	-	-	-		17	26	26	9
Treatment helping	N/A	91	81		95	93	91	100
Clinical Assessment								
Tenderness	1	1	1		1	1	1	1
Nodularity	2	1	1		1	1	1	1
Response	N/A	1	2		2	2	2	1
Prolactin mU/100 ml	13	-	17		14	40	-	6

PATIENT EV TRIAL NO. 9S (NON-CYCLICAL) GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	96	0	1		1	58	46	98
Breast Heaviness	15	0	7		1	-	-	-
Tenderness to touch	97	43	4		22	98	50	98
Sore feeling	98	0	3		0	97	-	98
Sex life	-	-	-		-	-	-	-
Nipple discharge	21	-	5		0	-	-	0
Headache	35	95	42		53	96	98	88
Feeling irritable	6	96	1		1	0	0	2
Feeling depressed	41	61	60		57	46	43	1
Bloated feeling	96	95	44		97	54	55	32
Well being	52	50	45		48	60	69	62
Feeling tired	-	-	95		97	99	98	97
Treatment helping	N/A	94	97		97	99	52	52
Clinical Assessment								
Tenderness	2	2	1		1	1	2	2
Nodularity	1	1	1		1	1	1	1
Response	N/A	3	1		1	3	2	2
Prolactin mU/100 ml	38	21	4		11	12	-	-

PATIENT BN TRIAL NO. 10S (NON-CYCLICAL) GROUP Bromocriptine -> Placebo DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	21	47	-		-	-	-	-
Breast Heaviness	9	0	-		-	-	-	-
Tenderness to touch	49	46	-		-	-	-	-
Sore feeling	8	13	-		-	-	-	-
Sex life	0	0	-		-	-	-	-
Nipple discharge	48	23	-		-	-	-	-
Headache	0	0	-		-	-	-	-
Feeling irritable	12	52	-		-	-	-	-
Feeling depressed	54	53	-		-	-	-	-
Bloated feeling	0	18	-		-	-	-	-
Well being	47	49	-		-	-	-	-
Feeling tired	0	9	-		-	-	-	-
Treatment helping	N/A	43	-		-	-	-	-
Clinical Assessment								
Tenderness	2	1	-		-	-	-	-
Nodularity	1	1	-		-	-	-	-
Response	N/A	3	-		-	-	-	-
Prolactin mU/100 ml	18	10	-		-	-	-	-

WITHDRAWAL IN CYCLE 1 DNA'd with side effects

PATIENT GJ TRIAL NO. 11S (NON-CYCLICAL) GROUP Bromocriptine -> Placebo DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	100	88	-		-	-	-	-
Breast Heaviness	15	5	-		-	-	-	-
Tenderness to touch	13	94	-		-	-	-	-
Sore feeling	100	94	-		-	-	-	-
Sex life	24	11	-		-	-	-	-
Nipple discharge	24	10	-		-	-	-	-
Headache	18	0	-		-	-	-	-
Feeling irritable	33	13	-		-	-	-	-
Feeling depressed	35	16	-		-	-	-	-
Bloated feeling	29	12	-		-	-	-	-
Well being	36	0	-		-	-	-	-
Feeling tired	100	88	-		-	-	-	-
Treatment helping	N/A	8	-		-	-	-	-
Clinical Assessment								
Tenderness	2	2	-		-	-	-	-
Nodularity	2	2	-		-	-	-	-
Response	N/A	3	-		-	-	-	-
Prolactin mU/100 ml	-	-	-		-	-	-	-

WITHDRAWAL IN CYCLE 1 - side effects (nausea/dizziness)

PATIENT JG TRIAL NO. 12S (NON-CYCLICAL) GROUP Placebo -> Bromocriptine DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	100	48	100		100	49	-	-
Breast Heaviness	48	100	100		0	100	-	-
Tenderness to touch	100	83	100		100	100	-	-
Sore feeling	100	84	100		100	100	-	-
Sex life	100	18	18		100	100	-	-
Nipple discharge	0	9	0		0	0	-	-
Headache	100	84	100		35	41	-	-
Feeling irritable	34	83	100		59	54	-	-
Feeling depressed	100	85	100		100	100	-	-
Bloated feeling	100	86	100		0	100	-	-
Well being	50	86	100		54	100	-	-
Feeling tired	100	87	100		69	100	-	-
Treatment helping	N/A	-	0		47	0	-	-
Clinical Assessment								
Tenderness	3	2	3		2	2	-	-
Nodularity	3	3	2		1	2	-	-
Response	N/A	3	3		2	2	-	-
Prolactin mU/100 ml	40	21	26		17	-	-	-

WITHDRAWAL IN CYCLE 4 (nausea/hypotension) side effects

PATIENT GH TRIAL NO. 13S (NON-CYCLICAL) GROUP Bromocriptine-> Placebo DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	69	52	-		-	-	-	-
Breast Heaviness	56	72	-		-	-	-	-
Tenderness to touch	100	40	-		-	-	-	-
Sore feeling	100	59	-		-	-	-	-
Sex life	100	57	-		-	-	-	-
Nipple discharge	47	1	-		-	-	-	-
Headache	78	59	-		-	-	-	-
Feeling irritable	98	100	-		-	-	-	-
Feeling depressed	79	88	-		-	-	-	-
Bloated feeling	74	54	-		-	-	-	-
Well being	82	84	-		-	-	-	-
Feeling tired	76	74	-		-	-	-	-
Treatment helping	N/A	88	-		-	-	-	-
Clinical Assessment								
Tenderness	2	2	-		-	-	-	-
Nodularity	3	3	-		-	-	-	-
Response	N/A	2	-		-	-	-	-
Prolactin mU/100 ml	5	<3	-		-	-	-	-

WITHDRAWAL IN CYCLE 2 - Side effects (Nausea/dizziness/depression/rash

PATIENT PR TRIAL NO. 14S (NON-CYCLICAL) GROUP BROMOCRIPTINE⟶ PLACEBO DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	59	75	70		85	86	85	87
Breast Heaviness	80	35	11		47	88	68	90
Tenderness to touch	83	100	90		88	88	87	89
Sore feeling	74	100	50		43	84	90	91
Sex life	48	100	84		84	82	81	85
Nipple discharge	3	7	9		10	9	9	9
Headache	40	35	37		37	34	13	9
Feeling irritable	38	96	89		83	85	48	54
Feeling depressed	76	51	61		50	85	50	19
Bloated feeling	81	10	88		85	88	85	86
Well being	47	45	67		66	45	55	47
Feeling tired	100	45	87		88	88	88	51
Treatment helping	N/A	3	6		8	9	9	9
Clinical Assessment								
Tenderness	2	2	2		3	3	3	3
Nodularity	3	3	2		2	1	2	2
Response	N/A	3	3		3	3	3	3
Prolactin mU/100 ml	3	13	56		6	8	5	15

PATIENT KB TRIAL NO. 15S (NON-CYCLICAL) GROUP Placebo -> Bromocriptine DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	97	73	53		-	-	-	-
Breast Heaviness	89	97	71		-	-	-	-
Tenderness to touch	88	98	98		-	-	-	-
Sore feeling	100	95	98		-	-	-	-
Sex life	32	95	98		-	-	-	-
Nipple discharge	7	2	0		-	-	-	-
Headache	3	1	0		-	-	-	-
Feeling irritable	98	98	98		-	-	-	-
Feeling depressed	98	98	98		-	-	-	-
Bloated feeling	99	2	0		-	-	-	-
Well being	49	97	97		-	-	-	-
Feeling tired	97	98	98		-	-	-	-
Treatment helping	N/A	2	93		-	-	-	-
Clinical Assessment								
Tenderness	3	2	1		-	-	-	-
Nodularity	3	3	3		-	-	-	-
Response	N/A	3	3		-	-	-	-
Prolactin mU/100 ml	14	-	4		-	-	-	-

WITHDRAWAL IN CYCLE 3 - Side effects (Hair loss)

PATIENT CN TRIAL NO. 16S GROUP PLACEBO → BROMOCRIPTINE DOSE 2.5 mg BD

VLA Score	Pre-trial	Cycle 1	Cycle 2		Cycle 3	Cycle 4	Cycle 5	Cycle 6
Pain	35	38	45		63	10	31	5
Breast Heaviness	2	5	0		-	0	-	0
Tenderness to touch	48	55	8		58	19	88	67
Sore feeling	28	62	49		56	1	22	1
Sex life	16	5	0		-	0	0	2
Nipple discharge	2	5	0		-	0	0	0
Headache	94	12	65		45	0	20	9
Feeling irritable	18	31	45		40	0	-	10
Feeling depressed	14	2	4		-	0	-	4
Bloated feeling	83	34	0		42	29	-	18
Well being	76	66	29		59	0	26	19
Feeling tired	77	82	33		70	21	30	63
Treatment helping	N/A	3	0		48	93	70	68
Clinical Assessment								
Tenderness	2	2	2		2	1	1	1
Nodularity	3	3	2		2	2	2	2
Response	N/A	3	3		2	2	2	2
Prolactin mU/100 ml	5	2	3		2	2	2	2

APPENDIX IV RAW DATA DANAZOL TRIAL

APPENDIX IV DANAZOL TRIAL RAW DATA

ENTRY CHARACTERISTICS

TRIAL NO	AGE (YRS)	CYCLE LENGTH (DAYS)	PAIN DURATION (YRS)
1	27	28	5
2	25	24	5
3	26	27	1
4	37	28	13
5	25	28	2
6	31	28	2
7	40	28	4
8	30	28	1.6
9	29	21	9
10	26	28	1
11	20	26	1.5
12	27	30	2.5
13	33	28	1
14	39	28	1.5
15	32	21	3
16	32	28	6
17	33	21	5
18	30	25	1
19	35	29	3
20	26	28	1
21	37	24	9
22	20	Irregular	6
23	30	28	13
24	37	24	5
25	22	30	1
26	Not entered	Not entered	Not entered
27	30	28	7
28	36	29	11
29	31	28	1

DANAZOL TRIAL RAW DATA

PATIENT M.S. TRIAL NO. 1 GROUP Placebo ⟶ Danazol DOSE 200 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	81	43	83	49		25	0	6
Discomfort	85	42	74	48		20	0	7
Feeling of well being	96	38	65	41		22	0	7
Irritability	95	24	92	90		46	41	35
Breast heaviness	51	24	62	37		23	0	7
Treatment helping	-	82	23	66		93	98	98
Tenderness	47	42	64	41		11	0	1
Depression	75	65	88	89		49	44	42
Soreness	49	45	74	43		18	0	2
Loss of sleep	45	65	92	83		94	21	2
Days of pain	16	13	23	14		16	6	5
Clinical Assessment								
Tenderness	2	3	3	2		2	1	1
Nodularity	3	2	2	1		1	1	1
Response to treatment	-	3	3	3		3	2	2
Weight kgs	58	60.5	56.5	56.7		55.8	55.5	57.6
Menses	Irreg	Irreg	Reg	Reg		None	None	Irreg

DANAZOL TRIAL RAW DATA

PATIENT J.C. TRIAL NO. 2 GROUP Placebo → Danazol DOSE 200 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	76	89	94	87		0	0	0
Discomfort	84	4	100	87		0	0	0
Feeling of well being	46	8	45	7		23	42	25
Irritability	46	7	7	4		22	0	0
Breast heaviness	95	90	100	87		22	1	0
Treatment helping	-	12	4	1		50	100	100
Tenderness	96	91	100	86		0	0	0
Depression	6	0	20	49		0	40	0
Soreness	96	90	100	87		0	0	0
Loss of sleep	6	0	0	0		0	0	0
Days of pain	14	15	13	17		9	0	0
Clinical Assessment								
Tenderness	2	2	3	2		1	1	1
Nodularity	3	3	2	2		2	2	2
Response to treatment	-	3	3	3		3	1	1
Weight kgs	47.5	47.8	46.5	46.2		48.4	48.4	47.8
Menses	Reg	Irreg	Reg	Reg		Reg	Reg	Reg

DANAZOL TRIAL RAW DATA

PATIENT P.T. TRIAL NO. 3 GROUP Danazol⟶ Placebo DOSE 200 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	35	23	96	11		30	25	21
Discomfort	79	22	89	6		25	17	14
Feeling of well being	26	29	95	11		16	26	15
Irritability	43	36	95	20		19	28	2
Breast heaviness	82	0	0	2		6	5	3
Treatment helping	--	36	0	2		5	61	65
Tenderness	78	5	0	3		27	7	10
Depression	9	25	76	5		18	8	2
Soreness	46	17	3	0		9	2	5
Loss of sleep	13	0	5	0		36	3	0
Days of pain	16	12	22	10		17	15	14
Clinical Assessment								
Tenderness	3	1	2	1		2	2	2
Nodularity	2	2	1	2		1	1	1
Response to treatment	-	2	3	2		2	2	2
Weight kgs	88.7	90.4	91.8	90.8		87.9	87.5	84.5
Menses	Reg	Irreg	None	Irreg		None	Reg	Reg

DANAZOL TRIAL RAW DATA

PATIENT R.J. TRIAL NO. 4 GROUP Placebo → Danazol DOSE 200 MG BI

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	52	100	4	3		0	2	3
Discomfort	93	100	3	1		2	3	1
Feeling of well being	93	11	6	2		0	2	0
Irritability	100	10	2	0		62	2	44
Breast heaviness	87	100	41	0		0	2	0
Treatment helping	-	4	28	100		100	100	100
Tenderness	23	100	3	9		0	2	0
Depression	9	60	3	1		45	60	0
Soreness	70	100	100	1		2	4	1
Loss of sleep	5	2	3	2		0	48	1
Days of pain	10	9	4	0		0	0	0
Clinical Assessment								
Tenderness	2	3	2	2		1	1	1
Nodularity	2	2	1	1		1	1	1
Response to treatment	-	3	2	1		1	1	1
Weight kgs	60.6	60.0	60.5	60.5		60.3	60.7	60.1
Menses	Reg	Reg	Reg	Reg		Irreg	Irreg	Irreg

DANAZOL TRIAL RAW DATA

PATIENT SO TRIAL NO. 5 GROUP DANAZOL -> PLACEBO DOSE 200mg BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	9	8	-	-	-	-	-	-
Discomfort	12	7	-	-		-	-	-
Feeling of well being	3	5	-	-		-	-	-
Irritability	58	6	-	-		-	-	-
Breast heaviness	14	4	-	-		-	-	-
Treatment helping	- -	6	-	-		-	-	-
Tenderness	10	5	-	-		-	-	-
Depression	56	5	-	-		-	-	-
Soreness	7	6	-	-		-	-	-
Loss of sleep	4	6	-	-		-	-	-
Days of pain	-	-	-	-		-	-	-
Clinical Assessment								
Tenderness	1	2	-	-		-	-	-
Nodularity	2	2	-	-		-	-	-
Response to treatment	N/A	1	-	-		-	-	-
Weight kgs	51.7	52	-	-		-	-	-
Menses	Reg	Reg	-	-		-	-	-

WITHDRAWAL ON BOTTLE 2 - FAILURE TO ATTEND

DANAZOL TRIAL RAW DATA

PATIENT K.A. TRIAL NO. 6 GROUP Placebo⟶ Danazol DOSE 200 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	42	54	95	65		1	2	2
Discomfort	80	32	90	63		32	4	2
Feeling of well being	42	18	45	43		34	71	2
Irritability	43	37	37	38		19	5	34
Breast heaviness	80	44	35	3		3	40	0
Treatment helping	-	42	9	31		28	88	96
Tenderness	96	76	94	33		31	0	2
Depression	4	3	12	50		1	1	2
Soreness	39	81	47	6		33	4	19
Loss of sleep	2	4	39	53		3	64	2
Days of pain	18	12	20	22		6	0	0
Clinical Assessment								
Tenderness	3	3	3	3		2	1	1
Nodularity	2	2	2	1		1	1	1
Response to treatment	-	3	3	3		2	1	1
Weight kgs	51	51	51.5	51.1		57.5	53.0	53.6
Menses	Reg	Reg	Reg	Reg		Reg	Irreg	Reg

DANAZOL TRIAL RAW DATA

PATIENT A.H. TRIAL NO. 7 GROUP Placebo → Danazol DOSE 100 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	74	12	5	86		22	52	2
Discomfort	77	11	8	92		13	3	2
Feeling of well being	77	41	65	19		51	97	3
Irritability	25	60	68	11		7	62	3
Breast heaviness	21	9	9	68		8	3	-
Treatment helping	--	19	42	9		21	92	100
Tenderness	87	24	23	97		8	4	3
Depression	6	29	16	99		7	86	3
Soreness	71	26	20	100		13	4	-
Loss of sleep	100	100	58	7		100	90	33
Days of pain	12	4	12	13		14	13	7
Clinical Assessment								
Tenderness	2	1	1	2		2	1	1
Nodularity	3	3	3	3		2	3	2
Response to treatment	-	2	3	3		2	4	2
Weight kgs	45.2	44.8	45.3	46		47.2	46.5	47.2
Menses	Reg	Reg	Reg	Reg		Irreg	Reg	Irreg

DANAZOL TRIAL RAW DATA

PATIENT V.B. TRIAL NO. 8 GROUP Danazol → Placebo DOSE 100 M 12

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	46	0	0	0		0	0	0
Discomfort	44	0	0	0		0	0	0
Feeling of well being	42	0	0	55		0	0	0
Irritability	0	0	0	0		0	0	0
Breast heaviness	15	0	0	0		0	0	0
Treatment helping		28	100	100		100	100	100
Tenderness	64	0	0	0		0	0	0
Depression	0	0	0	0		0	0	0
Soreness	30	0	0	0		0	0	0
Loss of sleep	0	0	0	0		0	0	0
Days of pain	10	0	0	0		0	0	7
Clinical Assessment								
Tenderness	1	1	1	1		1	1	1
Nodularity	3	2	2	2		1	1	2
Response to treatment		1	1	1		1	1	2
Weight kgs	60.1	62	62	N/K		62.6	62.2	61.7
Menses	Reg	Reg	None	None		None	Irreg	Reg

DANAZOL TRIAL RAW DATA

PATIENT LM TRIAL NO. 9 GROUP DANAZOL -> PLACEBO DOSE 400mgBD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	91	2	-	-		-	-	-
Discomfort	85	18	-	-		-	-	-
Feeling of well being	81	56	-	-		-	-	-
Irritability	56	52	-	-		-	-	-
Breast heaviness	97	21	-	-		-	-	-
Treatment helping	-	25	-	-		-	-	-
Tenderness	97	5	-	-		-	-	-
Depression	83	56	-	-		-	-	-
Soreness	59	3	-	-		-	-	-
Loss of sleep	2	3	-	-		-	-	-
Days of pain	-	-	-	-		-	-	-
Clinical Assessment								
Tenderness	2	1	-	-		-	-	-
Nodularity	2	3	-	-		-	-	-
Response to treatment	N/A	2	-	-		-	-	-
Weight kgs	55	55	-	-		-	-	-
Menses	Reg	Reg	-	-		-	-	-

WITHDRAWAL ON BOTTLE 2 - FAILURE TO ATTEND

DANAZOL TRIAL RAW DATA

PATIENT P.C. TRIAL NO. 10 GROUP Placebo → Danazol DOSE 100 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	43	42	8	13		5	5	2
Discomfort	98	35	7	15		10	14	2
Feeling of well being	40	36	60	0		25	39	30
Irritability	42	40	97	15		21	35	19
Breast heaviness	100	40	13	9		4	14	8
Treatment helping	-	0	34	75		95	-	93
Tenderness	100	44	17	41		4	20	3
Depression	44	41	99	14		31	63	24
Soreness	100	39	11	24		9	19	2
Loss of sleep	0	0	25	0		4	44	2
Days of pain	10	6	3	3		3	3	3
Clinical Assessment								
Tenderness	3	2	2	1		1	2	1
Nodularity	2	2	2	1		2	2	1
Response to treatment	-	3	2	1		1	4	1
Weight kgs	44.5	43.5	43.5	43.5		44.2	N/K	45.4
Menses	Reg	Reg	Irreg	Irreg		Irreg	Reg	Irreg

DANAZOL TRIAL RAW DATA

PATIENT J.E. TRIAL NO. 11 GROUP Placebo → Danazol DOSE 100 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	65	63	79	42		14	0	0
Discomfort	81	90	75	57		11	0	0
Feeling of well being	61	83	24	0		2	0	0
Irritability	83	72	55	39		4	0	0
Breast heaviness	84	85	72	39		31	9	0
Treatment helping	-	-	17	9		29	98	99
Tenderness	85	81	90	35		4	0	0
Depression	71	27	69	0		0	0	0
Soreness	69	73	89	29		5	0	0
Loss of sleep	23	0	0	0		0	0	0
Days of pain	14	9	15	10		5	0	0
Clinical Assessment								
Tenderness	2	2	2	2		1	1	1
Nodularity	2	2	2	3		2	1	1
Response to treatment	-	3	3	3		2	1	1
Weight kgs	53.5	53.3	54.2	51.6		53.4	55.3	55.8
Menses	Reg	Reg	Reg	Reg		Reg	Irreg	None

DANAZOL TRIAL RAW DATA

PATIENT B.W. TRIAL NO. 12 GROUP Danazol⟶ Placebo DOSE 100 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	9	100	0	0		18	47	0
Discomfort	3	100	0	0		14	45	0
Feeling of well being	78	22	0	34		0	0	0
Irritability	76	100	0	26		22	41	31
Breast heaviness	11	100	0	0		20	41	0
Treatment helping	-	0	-	100		100	100	100
Tenderness	9	100	0	0		15	37	0
Depression	80	76	0	21		17	41	0
Soreness	10	100	0	0		15	35	0
Loss of sleep	0	14	0	0		0	0	0
Days of pain	12	11	0	0		3	10	8
Clinical Assessment								
Tenderness	2	2	1	1		1	1	1
Nodularity	2	3	1	2		2	2	2
Response to treatment		3	1	1		4	1	3
Weight kgs	50.5	51.5	50.5	52		52.5	N/K	51.5
Menses	Reg	Reg	Irreg	Irreg		Irreg	Reg	Irreg

DANAZOL TRIAL RAW DATA

PATIENT CB TRIAL NO. 13 GROUP PLACEBO -> DANAZOL DOSE 100mg BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	30	46	-	-		-	-	-
Discomfort	34	38	-	-		-	-	-
Feeling of well being	23	70	-	-		-	-	-
Irritability	40	64	-	-		-	-	-
Breast heaviness	42	35	-	-		-	-	-
Treatment helping	-	-	-	-		-	-	-
Tenderness	33	25	-	-		-	-	-
Depression	18	61	-	-		-	-	-
Soreness	35	27	-	-		-	-	-
Loss of sleep	5	11	-	-		-	-	-
Days of pain	-	-	-	-		-	-	-
Clinical Assessment								
Tenderness	2	2	-	-		-	-	-
Nodularity	3	2	-	-		-	-	-
Response to treatment	N/A	3	-	-		-	-	-
Weight kgs	58.8	58.7	-	-		-	-	-
Menses	Reg	Irreg	-	-		-	-	-

WITHDRAWAL ON BOTTLE 1 - HEADACHES

DANAZOL TRIAL RAW DATA

PATIENT H.L. TRIAL NO. 14 GROUP Placebo⟶ Danazol DOSE 100 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	14	21	37	51		29	17	12
Discomfort	12	30	37	46		37	15	8
Feeling of well being	10	9	26	51		37	14	12
Irritability	45	48	52	61		51	23	25
Breast heaviness	41	44	47	64		54	13	14
Treatment helping	-	42	44	62		37	82	77
Tenderness	38	46	47	56		54	20	9
Depression	16	47	48	58		62	21	27
Soreness	35	40	45	46		53	14	7
Loss of sleep	82	60	28	56		34	13	5
Days of pain	12	13	12	14		13	9	5
Clinical Assessment								
Tenderness	1	1	1	1		1	1	1
Nodularity	2	2	2	2		1	1	1
Response to treatment	-	2	2	2		2	2	1
Weight kgs	52.2	58.2	60	52		59	59.5	61
Menses	Reg	Irreg	Reg	Reg		Irreg	Irreg	Reg

DANAZOL TRIAL RAW DATA

PATIENT J.C. TRIAL NO. 15 GROUP Placebo ⟶ Danazol DOSE 100 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	84	75	74	75		7	56	26
Discomfort	98	88	88	81		7	56	14
Feeling of well being	91	85	83	78		9	30	8
Irritability	34	8	7	44		8	26	6
Breast heaviness	86	81	89	74		9	27	13
Treatment helping		4	3	6		90	75	99
Tenderness	64	78	77	87		5	73	6
Depression	3	49	48	30		6	19	6
Soreness	97	96	94	84		5	45	6
Loss of sleep	94	98	95	96		89	83	92
Days of pain								
Clinical Assessment								
Tenderness	2	2	2	2		-	1	1
Nodularity	2	2	2	2		-	1	1
Response to treatment	-	3	3	3		2	2	1
Weight kgs	46.5	45.5	45.8	-		47.6	48.5	49.5
Menses	Reg	Reg	Reg	Reg		Reg	Irreg	None

DANAZOL TRIAL RAW DATA

PATIENT L.S. TRIAL NO. 16 GROUP Danazol ⟶ Placebo DOSE 100 MG BID

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	59	8	0	0		0	0	0
Discomfort	78	7	0	0		0	0	0
Feeling of well being	75	67	86	48		40	35	61
Irritability	74	74	88	36		38	34	59
Breast heaviness	78	2	0	0		0	0	0
Treatment helping		70	80	92		100	100	100
Tenderness	74	11	0	0		0	0	3
Depression	47	87	84	54		54	41	63
Soreness	45	8	0	0		1	0	0
Loss of sleep	48	16	43	32		64	43	79
Days of pain								
Clinical Assessment								
Tenderness	2	2	1	1		1	1	1
Nodularity	3	2	2	1		2	1	2
Response to treatment		2	1	1		1	1	1
Weight kgs	58.7	59.5	60.5	61.4		59.5	59	58
Menses	Reg	Irreg	Irreg	Irreg		Irreg	Reg	Reg

DANAZOL TRIAL RAW DATA

PATIENT D.J. TRIAL NO. 17 GROUP Danazol⟶ Placebo DOSE 100 MG BID

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	35	69	39	75		88	7	
Discomfort	91	88	43	68		92	7	
Feeling of well being	2	36	36	70		73	9	
Irritability	3	5	33	86		80	9	
Breast heaviness	84	93	41	88		92	4	
Treatment helping			55	77		19	94	
Tenderness	99	82	54	81		91	5	
Depression	2	14	46	88		70	5	
Soreness	89	77	38	89		90	5	
Loss of sleep	2	3	44	74		3	6	
Days of pain								
Clinical Assessment								
Tenderness	3	3	2	3		3	1	1
Nodularity	2	2	2	2		1	2	2
Response to treatment		3	3	3		3	2	2
Weight kgs	73.1	71.8		74		73.5	71.5	
Menses	Reg	Irreg	Reg	Reg		None	None	

DANAZOL TRIAL RAW DATA

PATIENT S.M. TRIAL NO. 18 GROUP Danazol⟶ Placebo DOSE [illegible]

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	65	80	0	0		0	0	0
Discomfort	97	100	0	0		44	0	[illegible]
Feeling of well being	12	100	0	0		25	0	[illegible]
Irritability	99	100	0	0		67	0	25
Breast heaviness	98	100	0	0		18	0	0
Treatment helping	-	0	83	100		79	100	100
Tenderness	98	100	0	0		0	0	0
Depression	32	100	0	0		33	0	34
Soreness	39	90	0	0		0	0	0
Loss of sleep	-	0	0	0		0	0	0
Days of pain	15	10	4	0		6	2	0
Clinical Assessment								
Tenderness	3	3	1	1		2	1	1
Nodularity	2	2	2	1		1	1	1
Response to treatment		3	1	1		2	1	1
Weight kgs	65.4	68.5	69.3	68.5		66	65.5	65.3
Menses	Reg	Irreg		Irreg		Irreg	Irreg	Reg

DANAZOL TRIAL RAW DATA

PATIENT V.H. TRIAL NO. 19 GROUP Placebo⟶ Danazol DOSE 100 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	94	96	98	32		18	4	2
Discomfort	94	97	99	40		22	4	3
Feeling of well being	27	10	46	14		55	20	-
Irritability	99	96	97	15		35	27	2
Breast heaviness	95	98	99	44		22	4	5
Treatment helping		7	3	71		74	100	100
Tenderness	100	99	100	65		61	4	3
Depression	91	99	52	11		11	17	61
Soreness	100	98	100	72		51	3	3
Loss of sleep	99	97	100	10		19	4	3
Days of pain								
Clinical Assessment								
Tenderness	3	3	3	2		2	1	1
Nodularity	2	1	1	1		1	1	1
Response to treatment	-	3	3	2		2	1	-
Weight kgs	53.6	57	56.4	56		57.3	57.5	
Menses	Reg	Irreg	Reg	Reg		Reg	Irreg	Irreg

DANAZOL TRIAL RAW DATA

PATIENT MR TRIAL NO. 20 GROUP PLACEBO -> DANAZOL DOSE 100mg BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	58	80	65	-		-	-	-
Discomfort	98	71	69	-		-	-	-
Feeling of well being	93	59	96	-		-	-	-
Irritability	97	81	89	-		-	-	-
Breast heaviness	99	19	88	-		-	-	-
Treatment helping	-	72	14	-		-	-	-
Tenderness	91	77	93	-		-	-	-
Depression	13	74	97	-		-	-	-
Soreness	94	47	81	-		-	-	-
Loss of sleep	98	9	54	-		-	-	-
Days of pain	-	-	-	-		-	-	-
Clinical Assessment								
Tenderness	3	3	3	-		-	-	-
Nodularity	1	1	1	-		-	-	-
Response to treatment	N/A	3	3	-		-	-	-
Weight kgs	69.2	71.4	-	-		-	-	-
Menses	Reg	Reg	Reg	-		-	-	-

WITHDRAWAL ON BOTTLE 2 - FAILURE TO ATTEND

DANAZOL TRIAL RAW DATA

PATIENT B.H. TRIAL NO. 21 GROUP Danazol → Placebo DOSE 100 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	53	9	59	9		15	12	17
Discomfort	65	22	44	11		16	16	12
Feeling of well being	60	46	37	13		15	42	15
Irritability	70	71	17	12		22	13	14
Breast heaviness	11	9	13	13		7	12	7
Treatment helping			14	79		77	76	88
Tenderness	49	14	36	7		12	61	67
Depression	14	52	12	9		17	19	21
Soreness	8	15	9	10		15	13	20
Loss of sleep	4	9	9	6		14	15	15
Days of pain								
Clinical Assessment								
Tenderness	3	1	2	1		2	2	3
Nodularity	3	2	2	1		2	3	3
Response to treatment		2	3	1		2	2	3
Weight kgs	61	64.1	67.2	67.5		63	63.5	64
Menses	Reg	Irreg	None	Reg		Irreg	Reg	Reg

DANAZOL TRIAL RAW DATA

PATIENT SD TRIAL NO. 22 GROUP DANAZOL -> PLACEBO DOSE 100mg BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	62	27	-	-		-	-	-
Discomfort	76	37	-	-		-	-	-
Feeling of well being	8	55	-	-		-	-	-
Irritability	75	35	-	-		-	-	-
Breast heaviness	19	15	-	-		-	-	-
Treatment helping	-	50	-	-		-	-	-
Tenderness	99	73	-	-		-	-	-
Depression	76	52	-	-		-	-	-
Soreness	11	59	-	-		-	-	-
Loss of sleep	10	4	-	-		-	-	-
Days of pain	-	-	-	-		-	-	-
Clinical Assessment								
Tenderness	1	1	-	-		-	-	-
Nodularity	2	3	-	-		-	-	-
Response to treatment	N/A	3	-	-		-	-	-
Weight kgs	56	56	-	-		-	-	-
Menses	Irreg	Irreg	-	-		-	-	-

WITHDRAWAL ON BOTTLE 1 - ? PREGNANT

DANAZOL TRIAL RAW DATA

PATIENT RC TRIAL NO. 23 GROUP DANAZOL -> PLACEBO DOSE [illegible]

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	94	86	80	58		92	35	[illegible]
Discomfort	94	80	80	59		90	83	59
Feeling of well being	88	92	68	73		5	32	85
Irritability	86	93	71	93		82	94	95
Breast heaviness	84	74	52	17		88	86	[illegible]
Treatment helping	-	75	27	45		16	47	35
Tenderness	85	92	85	73		87	71	76
Depression	29	92	91	77		11	96	97
Soreness	84	90	45	79		80	48	[illegible]
Loss of sleep	5	15	10	8		5	6	4
Days of pain		15	16	1		17	17	16
Clinical Assessment								
Tenderness	3	3	3	3		2	3	3
Nodularity	2	2	2	1		3	2	2
Response to treatment	N/A	3	2	3		4	3	3
Weight kgs	60	62.1	61.6	61.5		61.5	60.5	[illegible]
Menses	Reg.	Irreg.	None	Irreg.		None	Irreg.	Irreg.

DANAZOL TRIAL RAW DATA

PATIENT J.J. TRIAL NO. 24 GROUP Placebo → Danazol DOSE 100 MG BID

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	3	24	64	25		12	3	6
Discomfort	92	23	75	19		8	4	3
Feeling of well being	29	50	51	24		5	3	-
Irritability	90	63	59	69		41	16	22
Breast heaviness	90	13	71	25		11	2	2
Treatment helping	-	70	60	84		93	97	96
Tenderness	100	53	89	25		12	2	2
Depression	45	24	68	30		43	15	9
Soreness	98	51	90	45		11	2	1
Loss of sleep	60	58	73	15		39	14	14
Days of pain								
Clinical Assessment								
Tenderness	3	2	3	3		1	1	1
Nodularity	2	2	2	2		2	1	2
Response to treatment	-	2	3	3		2	1	1
Weight kgs	61.5	61.5	63.3	62.3		65.8	65.2	63
Menses	Reg	Reg	-	Reg		Reg	Reg	Irreg

DANAZOL TRIAL RAW DATA

PATIENT K.F. TRIAL NO. 25 GROUP Danazol → Placebo DOSE 100 MG BID

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	59	14	41	33		20	10	10
Discomfort	64	14	40	43		21	11	8
Feeling of well being	28	5	20	16		19	14	2
Irritability	41	40	43	39		32	12	8
Breast heaviness	66	42	60	53		17	12	8
Treatment helping	N/A	85	82	78		81	91	97
Tenderness	85	44	49	63		21	24	85
Depression	10	15	16	10		21	8	6
Soreness	89	44	52	72		25	11	93
Loss of sleep	9	4	4	4		13	9	2
Days of pain								
Clinical Assessment								
Tenderness	2	1	1	2		2	0	2
Nodularity	3	2	2	2		2	0	2
Response to treatment		1	2	3		2	1	2
Weight kgs	42.0	44.0	44.6	44.5				42.0
Menses	Reg	Irreg	Irreg	None		Reg	Reg	Reg

DANAZOL TRIAL RAW DATA

PATIENT P.L. TRIAL NO. 27 GROUP Placebo -> Danazol DOSE [illegible]

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	80	80	47	75		10	4	21
Discomfort	94	45	70	82		15	5	21
Feeling of well being	100	84	30	83		27	3	30
Irritability	100	77	77	87		27	29	21
Breast heaviness	100	67	65.	90		17	5	12
Treatment helping	N/A	30	45	15		76	94	79
Tenderness	100	43	56	96		32	5	20
Depression	69	81	73	94		20	17	13
Soreness	84	65	43	91		10	2	3
Loss of sleep	49	94	69	12		60	87	72
Days of pain								
Clinical Assessment								
Tenderness	2	2	2	3		1	1	2
Nodularity	2	3	3	2		1	1	1
Response to treatment	-	3	3	3		1	1	2
Weight kgs	54.6	54.0	53.25	53.25		53.0	53.5	53.25
Menses		Reg		Reg		Irreg	Irreg	Irreg

DANAZOL TRIAL RAW DATA

PATIENT LT TRIAL NO. 28 GROUP PLACEBO -> DANAZOL DOSE 100mg BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	100	0	70	-		-	-	-
Discomfort	100	0	66	-		-	-	-
Feeling of well being	93	0	14	-		-	-	-
Irritability	100	0	42	-		-	-	-
Breast heaviness	100	45	65	-		-	-	-
Treatment helping	-	96	4	-		-	-	-
Tenderness	100	0	65	-		-	-	-
Depression	0	0	52	-		-	-	-
Soreness	100	0	49	-		-	-	-
Loss of sleep	75	55	74	-		-	-	-
Days of pain	-	-	-	-		-	-	-
Clinical Assessment								
Tenderness	2	2	2	-		-	-	-
Nodularity	3	1	1	-		-	-	-
Response to treatment	N/A	3	3	-		-	-	-
Weight kgs	69.5	70	70	-		-	-	-
Menses	Reg	Reg	Reg	-		-	-	-

WITHDRAWAL ON BOTTLE 2 - SIDE EFFECTS

DANAZOL TRIAL RAW DATA

PATIENT B.H. TRIAL NO. 29 GROUP Placebo → Danazol DOSE 100 MG BD

VLA Score	Pre-trial	Bottle 1	Bottle 2	Bottle 3		Bottle 4	Bottle 5	Bottle 6
Pain	69	33	11	10		66	2	2
Discomfort	92	25	38	5		57	2	2
Feeling of well being	52	92	2	12		2	2	2
Irritability	69	25	4	9		2	2	2
Breast heaviness	58	15	2	3		84	2	3
Treatment helping	N/A	-	97	83		5	98	2
Tenderness	74	84	3	7		70	5	98
Depression	3	7	1	2		1	3	11
Soreness	80	39	2	13		85	2	2
Loss of sleep	15	2	2	5		2	2	7
Days of pain	16	18	12	10		17	7	4
Clinical Assessment								
Tenderness	2	2	1	1		2	1	1
Nodularity	1	2	1	2		2	1	1
Response to treatment	-	3	2	2		2	1	1
Weight kgs	59.0	59.5	58.5	-		59.0	-	
Menses	Reg	Reg	Irreg	-		Irreg	Irreg	

DANAZOL TRIAL - PROGESTERONE RESULTS

Placebo First

Patient No.	Pre-trial	1	2	3	4	5	6
1	M	38.0	7.3	28.0	1.3	1.3	1.1
2	4.3	23.0	48.0	51.0	2.1	3.2	3.1
4	32.0	32.0	51.0	2.4	1.4	20.0	9.0
6	45.0	51.0	10.0	44.0	1.9	<0.5	1.1
7	14.0	25.0	19.5	17.0	1.0	0.8	0.7
10	2.3	31.0	38.0	14.0	M	0.7	<0.5
24	32.0	35.0	38.0	M	10.8	6.9	<0.5
27	2.2	1.7	22.0	3.4	1.5	1.1	1.1
29	M	38.0	2.8		2.4	11.0	M
n	7	9	9	7	8	9	8
Mean	18.3	30.52	26.29	22.83	2.80	5.06	2.14
Std.Deviation	17.40	13.56	18.02	19.04	3.26	6.64	2.90
Std.Error	6.58	4.52	6.01	7.20	1.15	2.21	1.03

Danazol First

3	8.3	1.4	1.4	1.9	9.5	15.5	18.5
8	29.0	1.4	1.3	1.1	20.2	19.0	23.5
12	50.0	1.2	0.7	0.9	<0.5	31.0	M
16	27.0	0.7	<0.5	1.2	6.7	M	3.8
21	M	1.4	6.2	1.8	1.3	8.5	M
23	M	2.2	M	M	20.0	1.3	<0.5
25	6.4	1.1	M	1.0	<0.5	1.8	22.3
Mean	24.14	1.34	2.02	1.32	8.39	12.85	13.72
Std.Deviation	17.79	0.45	2.37	0.43	8.69	11.39	10.79
Std.Error	7.96	0.17	1.06	0.18	3.28	4.65	4.83

M = Missing

DANAZOL TRIAL - PROLACTIN RESULTS

Placebo First

Patient No.	Pre-trial	1	2	3	4	5	6
1	M	M	M	M	M	M	M
2	M	M	M	M	M	M	M
4	160	143	242	226	163	134	186
6	216	195	212	134	134	209	163
7	63	114	152	174	63	143	63
10	258	163	246	103	M	125	152
24	M	M	M	M	M	M	M
27	M	M	M	M	M	M	M
29	M	M	M	M	M	M	M
n	4	4	4	4	3	4	4
Mean	174.25	154.50	213.00	159.25	120.00	152.75	141.00
Std.Deviation	84.33	32.92	43.41	53.15	51.45	38.21	53.89
Std.Error	42.17	16.46	21.71	26.58	29.70	19.11	26.95

Danazol First

3	263	63	242	150	307	315	331
8	186	226	174	258	226	226	246
12	171	174	236	174	186	258	M
16	134	174	143	150	186	M	263
21	M	M	M	M	M	M	M
23	M	M	M	M	M	M	M
25	174	195	M	125	152	212	212
n	5	5	4	5	5	4	4
Mean	185.60	165.40	198.75	171.40	211.40	252.75	263.00
Std.Deviation	47.44	61.60	48.23	51.42	59.52	45.75	50.05
Std.Error	21.22	27.55	24.12	23.00	26.62	22.88	25.03

M = Missing

DANAZOL TRIAL - OESTRADIOL RESULTS

Placebo First

Patient No.	Pre-trial	1	2	3	4	5	6
1	M	620	720	430	1700	860	1800
2	<50	460	570	210	1650	2050	1650
4	950	970	750	220	1500	1500	1600
6	1050	1050	540	650	2300	390	1560
7	540	570	460	620	1600	1450	2000
10	350	880	720	480	M	1800	1140
24	800	420	600	M	1220	1650	1570
27	250	60	310	80	1850	1130	1130
29	M	420	180		970	1000	M
n	7	9	9	7	8	9	8
Mean	570.00	605.56	538.89	384.29	1598.75	1314.44	1566.25
Std.Deviation	376.34	315.20	194.39	218.85	399.34	516.75	308.63
Std.Error	142.24	105.07	64.8	82.72	141.20	172.25	109.12

Danazol First

3	540	1600	1350	880	160	360	250
8	485	1300	1600	1300	285	350	425
12	370	1580	370	1250	92	440	M
16	640	1300	930	1250	300	M	130
21	M	1340	950	1600	680	175	M
23	M	1000	M	M	220	115	500
25	155	1670	M	1900	120	330	430
Mean	438	1398.57	1040	1363.33	265.29	295.00	347.00
Std.Deviation	185.83	234.12	468.72	348.52	199.10	123.53	152.38
Std.Error	83.11	88.49	209.62	142.28	75.25	50.43	68.15

M = Missing

APPENDIX V RAW DATA EPO TRIAL

Patients' Registation Details

Pat No	Date of Birth	Date of Entry	Date Completed	Hospital No.	Sequence	(a) Pain	Dur Yrs	Site	(b) Nod	(b) M	Comments	(c) Pit	Comments	Age
01	30-11-43	13- 4-82	28- 7-82	A250895T	Efamol	1	1.0	L Breast	N	Y	N	Y	*	38
03	29-11-29	27- 4-82	7- 9-82	A239450E	Efamol	2	26.0	R Breast	Y	Y	DY	N	*	52
05	27- 2-56	27- 4-82	10- 8-82	A433111L	Efamol	1	0.5	Upper quadrants R>L	Y	Y	B (no coding)	N	*	26
06	30-12-48	4- 5-82	24- 8-82	A434228V	Efamol	1	5.0	U.O.Q's	Y	N	*	N	*	33
09	11-10-46	3- 6-82	21- 9-82	A199443W	Efamol	1	0.6	L outer half	Y	Y	P2G	N	*	35
14	16- 7-55	13- 7-82	2-11-82	A438063E	Efamol	3	1.2	L>>R	N	Y	P1A	N	*	26
16	28- 2-59	10- 8-82	30-11-82	A433459L	Efamol	2	1.0	L>>R outer half	N	N	*	N	*	23
17	8- 1-43	10- 8-82	23-11-82	A360291M	Efamol	1	4.0	L=R outer halves	Y	Y	P2M	N	*	39
18	4- 3-45	7- 9-82	21-12-82	A395214X	Efamol	1	2.0	U.O.Q.'s	Y	Y	DY	N	*	37
20	8- 8-50	14- 9-82	14- 9-82	A256521E	Efamol	1	0.5	Inframammary	Y	Y	DY	N	*	32
21	11- 3-62	14- 9-82	28-12-82	A438346K	Efamol	1	0.8	Outer halves	Y	N	*	N	*	20
24	17- 6-49	5-10-82	1- 2-83	A114317W	Efamol	*	*	*	*	Y	B	N	*	33
25	9- 7-55	5-10-82	2-11-82	A437431F	Efamol	1	0.8	Outer halves L>R	Y	N	*	N	*	27
28	7- 3-46	12-10-82	7-12-82	A426355S	Efamol	1	*	L>R U.O.Q. and upper half	Y	N	*	N	*	36
30	6- 1-45	26-10-82	23-11-82	A197008M	Efamol	2	2.0	L breast, nipple, areola - U.O.Q.	N	N	*	N	*	37
33	15-12-31	30-11-82	22- 3-83	A024527S	Efamol	1	1.0	U.O.Q.'s	Y	Y	P2G	N	*	50
34	11- 5-51	21-12-82	10- 5-83	A439892P	Efamol	1	3.0	U.O.Q.'s	Y	N	*	N	*	31
36	8-12-48	1- 3-83	5- 7-83	A193289F	Efamol	1	2.0	U.O.Q.'s	Y	Y	1975 Benign	N	*	34
38	30- 8-51	10- 5-83	13- 9-83	A434839V	Efamol	1	1.5	*	Y	N	*	N	*	31
39	23-10-58	7- 6-83	27- 9-83	A345379E	Efamol	1	1.0	U.O.Q.'s	Y	N	*	N	*	24
42	8- 7-44	2- 8-83	22-11-83	A043347L	Efamol	1	3.0	U.O.Q.'s mainly	Y	Y	DY	N	*	39
44	25- 7-53	25-10-83	14- 2-84	A033719V	Efamol	1	1.0	L breast U.O.Q.	Y	Y	B	N	*	30
45	16-12-50	1-11-83	14- 2-84	A345809D	Efamol	1	2.0	All over - worse U.O.Q.'s R>L	Y	Y	DY	N	*	32
46	25- 5-51	6-12-83	3- 4-84	A352552F	Efamol	1	2.5	U.O.Q.'s	N	N	*	N	*	32
48	30- 8-58	17- 1-84	8- 5-84	A479767U	Efamol	1	1.0	U.I.Q. L	Y	N	*	N	*	25
02	12- 7-37	22- 4-82	3- 8-82	A068413N	Placebo	1	0.6	UOQ and upper half	N	Y	Y	Y	*	44
04	25- 7-43	27- 4-82	17- 8-82	A400259P	Placebo	1	20.0	Both breasts behind nipple	Y	Y	P2G	N	*	38
07	5- 2-47	11- 5-82	1- 9-82	A351224H	Placebo	1	1.0	Inframammary	N	Y	P1A	N	*	35
08	26- 7-41	18- 5-82	1- 9-82	A427121X	Placebo	1	2.0	U.O.Q's Inframammary	Y	N	*	N	*	40
10	2- 8-40	12- 7-83	25-10-83	A132185F	Placebo	1	2.0	UOQ's and nipplesL>R	N	Y	P2M	N	*	42
11	11-10-56	6- 7-82	9-11-82	A401442C	Placebo	1	1.0	Inframammary L>R	Y	Y	B (25 years)	N	*	25
15	3-11-55	27- 7-82	16-11-82	A277863R	Placebo	3	3.5	U.O.Q. R>L	Y	N	*	N	*	26
19	13-12-50	14- 9-82	18- 1-83	A232785U	Placebo	1	3.0	Outer halves	Y	N	*	N	*	31
22	21- 6-43	21- 9-82	8- 2-83	A287794C	Placebo	1	8.0	Outer halves	Y	Y	P2G	N	*	39
23	23- 6-41	21- 9-82	21- 9-82	A099127X	Placebo	2	2.0	Outer halves R>L	Y	Y	DY	N	*	41
26	3- 6-54	5-10-82	18- 1-83	A333427M	Placebo	*	3.0	Outer halves	Y	N	*	N	*	28
27	27- 3-61	12-10-82	25- 1-83	A299216Y	Placebo	1	*	*	Y	N	*	N	*	21
29	22- 1-40	26-10-82	1- 2-83	A423887C	Placebo	1	22.0	L=R Nipple - U.O.Q.'s	Y	Y	P2G	N	*	42
31	13-10-54	11-11-82	1- 3-83	A058133D	Placebo	1	8.0	Bilat. L=R U.O.Q.'s and inframammary	Y	N	*	N	*	28
32	8- 7-57	23-11-82	15- 3-83	A452039N	Placebo	3	0.3	L>R U.O.Q - nipple	Y	N	*	N	*	25
35	15- 6-46	1- 2-83	24- 5-83	A282800J	Placebo	1	10.0	U.O.Q. R>L	Y	N	*	N	*	36
37	23- 5-44	26- 4-83	9- 8-83	A256994J	Placebo	1	1.0	L>R outer halves	Y	Y	DY	N	*	38
40	5- 9-56	28- 6-83	8-11-83	A369834H	Placebo	1	4.0	U.O.Q.'s L>R	Y	N	*	N	*	26
41	14-11-49	19- 7-83	1-11-83	A325087J	Placebo	1	1.5	U.O.Q.'s	Y	Y	P2G	N	*	33
43	21- 8-52	25-10-83	14- 2-84	A119031D	Placebo	1	1.5	U.O.Q. L breast	N	N	*	N	*	31
47	24-12-54	10- 1-84	1- 5-84	A361335P	Placebo	1	2.0	U.O.Q.'s	N	Y	B	N	*	29

(a) Pain 1= Cyclical, 2= Non cyclical irregular and 3= Non cyclical contiuous.
(b) M= Mammogram.
(c) Pit= Pituitary Function Test.

Pat No	Sequence	Pain 0	Pain 1	Pain 2	Pain 3	Pain 4	Tenderness to Touch- 0	Tenderness to Touch- 1	Tenderness to Touch- 2	Tenderness to Touch- 3	Tenderness to Touch- 4	Breast Heaviness 0	Breast Heaviness 1	Breast Heaviness 2	Breast Heaviness 3	Breast Heaviness 4	Lumpiness 0	Lumpiness 1	Lumpiness 2	Lumpiness 3	Lumpiness 4
01	Efamol	67	81	29	22	6	80	97	60	73	10	80	73	75	62	76	*	6	10	21	17
03	Efamol	67	48	30	10	10	78	60	61	25	41	4	3	3	2	3	2	3	4	2	2
05	Efamol	*	38	24	*	30	*	38	29	*	20	*	38	23	*	15	*	44	28	*	26
06	Efamol	55	72	96	*	93	88	67	95	*	92	90	72	96	*	93	90	87	97	*	93
09	Efamol	66	71	72	79	84	77	63	90	77	82	61	43	63	70	80	81	59	63	75	90
14	Efamol	98	74	83	86	97	99	94	97	98	90	72	70	86	76	76	50	64	66	75	74
16	Efamol	75	68	73	85	72	45	17	62	61	37	6	2	3	23	6	5	2	3	3	2
17	Efamol	75	87	94	96	94	92	83	96	99	92	91	83	97	99	93	33	73	95	95	92
18	Efamol	98	55	8	*	16	98	57	41	*	17	99	56	7	*	9	99	54	66	*	53
20	Efamol	86	*	*	*	*	87	*	*	*	*	80	*	*	*	*	18	*	*	*	*
21	Efamol	45	61	95	98	20	88	72	100	98	12	44	48	76	58	0	69	50	71	73	5
24	Efamol	48	96	8	7	2	94	92	5	5	2	90	70	5	2	2	70	68	19	14	2
25	Efamol	69	28	*	*	*	72	23	*	*	*	82	31	*	*	*	80	35	*	*	*
28	Efamol	94	*	36	*	*	82	*	41	*	*	2	*	2	*	*	91	*	86	*	*
30	Efamol	93	22	*	*	*	91	39	*	*	*	5	9	*	*	*	7	9	*	*	*
33	Efamol	61	88	82	80	55	91	92	95	95	51	92	96	84	82	50	90	92	92	85	53
34	Efamol	95	95	93	93	94	95	95	92	90	91	94	93	92	89	89	93	*	90	89	88
36	Efamol	90	44	48	54	52	89	79	52	82	84	88	71	81	85	80	86	75	80	62	54
38	Efamol	95	5	5	5	6	92	6	93	71	5	93	8	5	4	5	48	7	7	5	5
39	Efamol	78	10	66	80	86	99	11	87	91	86	98	13	42	83	88	97	16	70	92	81
42	Efamol	88	85	80	85	85	91	86	83	86	87	89	88	85	89	88	89	87	85	87	85
44	Efamol	76	52	16	10	54	83	50	23	16	32	68	43	6	3	33	67	49	23	20	28
45	Efamol	95	93	90	69	17	98	94	91	96	68	2	48	61	49	7	98	49	42	46	70
46	Efamol	75	64	85	41	80	93	94	77	77	57	80	80	98	80	91	*	32	43	29	15
48	Efamol	73	79	64	15	16	92	85	67	12	24	80	75	58	18	9	6	62	48	6	12
02	Placebo	3	4	2	64	*	98	8	1	80	*	15	2	1	4	*	3	2	1	2	*
04	Placebo	*	82	61	*	72	*	87	56	*	78	*	87	11	*	56	*	88	12	*	25
07	Placebo	78	*	44	18	12	86	*	34	17	17	87	*	35	10	13	2	*	0	0	0
08	Placebo	64	82	80	55	6	61	94	87	73	6	99	98	93	95	36	98	97	67	72	35
10	Placebo	62	94	90	76	88	96	92	87	3	56	96	66	87	55	80	1	1	17	3	8
11	Placebo	68	57	61	40	65	90	75	65	40	65	83	68	55	35	*	78	80	48	35	56
15	Placebo	3	22	1	*	14	94	51	10	*	12	95	46	1	*	6	92	41	12	*	9
19	Placebo	97	95	77	96	97	97	96	78	97	97	79	96	81	97	97	50	*	*	*	*
22	Placebo	79	90	5	26	22	80	92	42	27	18	81	94	40	27	31	82	93	37	30	28
23	Placebo	92	56	73	*	*	90	60	87	*	*	92	57	25	*	*	91	27	70	*	*
26	Placebo	74	93	96	*	95	75	92	94	*	99	100	90	96	*	99	99	87	98	*	96
27	Placebo	5	54	0	49	50	100	100	87	100	100	100	100	86	100	100	70	100	85	100	100
29	Placebo	87	96	*	97	85	86	97	*	97	98	86	97	*	80	97	24	91	*	97	98
31	Placebo	98	36	*	43	2	94	36	*	43	9	71	46	*	48	4	93	47	*	37	7
32	Placebo	76	94	83	65	24	92	99	97	71	36	91	96	97	71	22	65	90	71	42	22
35	Placebo	88	88	100	100	31	88	100	100	100	26	72	94	100	100	23	82	94	100	100	22
37	Placebo	92	82	85	91	95	91	95	96	93	96	90	92	95	94	95	90	95	94	94	95
40	Placebo	75	71	61	51	75	84	81	61	49	82	50	66	41	39	76	65	69	46	42	73
41	Placebo	83	60	82	75	27	96	63	82	80	27	40	23	55	69	47	78	46	71	73	31
43	Placebo	58	59	84	71	95	84	81	97	74	94	47	51	56	72	75	84	35	37	59	69
47	Placebo	98	61	76	35	68	98	63	92	73	82	98	67	99	72	76	*	2	7	1	5

Pat No	Sequence	-- Irritability ---- 0	1	2	3	4	Feeling of Well Being 0	1	2	3	4	-Is Treatment Helping - 0	1	2	3	4
01	Efamol	82	91	86	64	7	86	92	62	67	10	*	3	45	27	87
03	Efamol	17	15	7	2	5	3	7	5	3	2	*	16	50	85	48
05	Efamol	*	37	29	*	45	*	23	13	*	45	*	3	24	*	25
06	Efamol	19	77	96	*	93	10	71	97	*	52	*	49	9	*	3
09	Efamol	96	81	73	63	82	56	22	46	46	62	*	44	17	19	8
14	Efamol	99	81	89	97	85	50	65	91	93	48	*	2	2	2	2
16	Efamol	94	97	74	54	31	48	23	62	62	32	*	46	52	57	53
17	Efamol	93	97	25	99	99	94	94	47	99	92	*	41	60	19	58
18	Efamol	87	4	39	*	0	100	4	39	*	2	*	*	57	*	69
20	Efamol	54	*	*	*	*	50	*	*	*	*	*	*	*	*	*
21	Efamol	94	70	80	100	17	40	48	76	96	21	*	0	0	0	0
24	Efamol	93	39	1	2	2	93	29	2	4	5	*	43	88	71	98
25	Efamol	50	6	*	*	*	62	51	*	*	*	*	60	*	*	*
28	Efamol	90	*	86	*	*	42	*	49	*	*	*	*	85	*	*
30	Efamol	94	43	*	*	*	94	10	*	*	*	*	92	*	*	*
33	Efamol	14	97	95	80	51	78	96	94	88	39	*	0	20	90	48
34	Efamol	94	93	91	87	87	93	94	92	91	87	*	2	*	88	7
36	Efamol	51	50	50	47	48	50	46	50	44	50	*	21	11	16	14
38	Efamol	90	6	50	7	5	9	9	6	2	7	*	91	96	86	93
39	Efamol	40	10	44	71	66	24	17	22	42	42	*	82	67	54	25
42	Efamol	74	67	84	55	51	62	70	54	41	51	*	5	7	9	6
44	Efamol	83	74	36	17	15	57	77	19	16	6	*	*	69	75	81
45	Efamol	62	71	96	49	48	46	46	80	51	17	*	6	46	11	42
46	Efamol	88	29	45	25	65	73	48	45	34	27	*	59	40	79	7
48	Efamol	48	76	48	20	15	49	51	22	40	50	*	2	5	78	99
02	Placebo	79	1	1	2	*	18	1	1	3	*	*	98	99	24	*
04	Placebo	*	19	8	*	45	*	19	7	*	45	*	9	24	*	14
07	Placebo	82	*	42	0	18	80	*	46	10	24	*	*	52	53	84
08	Placebo	98	95	56	82	1	89	55	72	86	1	*	2	35	*	99
10	Placebo	31	85	75	10	75	92	59	66	79	50	*	41	34	42	36
11	Placebo	0	0	0	0	0	0	0	0	0	0	*	0	23	40	35
15	Placebo	52	83	6	*	6	82	80	12	*	13	*	69	98	*	85
19	Placebo	71	96	82	98	96	54	61	86	94	50	*	6	5	3	4
22	Placebo	94	93	37	18	27	45	52	37	42	37	*	7	87	93	97
23	Placebo	92	36	75	*	*	93	47	57	*	*	*	51	40	*	*
26	Placebo	96	89	71	*	87	73	79	82	*	91	*	2	2	*	0
27	Placebo	24	0	0	0	51	0	0	0	0	0	*	0	43	0	0
29	Placebo	97	98	*	97	98	85	98	*	98	82	*	4	*	24	8
31	Placebo	62	53	*	43	1	40	20	*	4	62	*	3	*	85	86
32	Placebo	97	100	89	40	24	96	96	89	45	24	*	*	92	96	98
35	Placebo	82	73	100	100	19	48	72	100	100	26	*	0	0	14	61
37	Placebo	88	95	95	95	95	86	84	92	93	94	*	7	3	2	3
40	Placebo	65	36	38	34	27	38	23	47	25	27	*	*	1	24	4
41	Placebo	100	52	68	52	32	81	21	82	50	34	*	*	9	60	72
43	Placebo	98	84	74	68	80	43	28	47	42	51	*	27	5	18	30
47	Placebo	95	88	89	50	94	69	78	70	48	87	*	2	4	10	4

Pat No	Sequence	Tenderness					Nodularity					Treatment Help					Weight				
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
01	Efamol	3	3	2	2	1	1	2	2	1	1	*	3	2	2	1	*	*	60.0	63.4	*
03	Efamol	3	3	3	3	2	2	2	1	1	2	*	3	2	2	2	*	*	64.2	64.7	64.0
05	Efamol	2	2	2	*	2	2	2	3	*	2	*	0	3	*	2	*	50.2	50.9	*	50.7
06	Efamol	3	*	2	*	1	3	*	3	*	3	*	*	3	*	3	66.0	*	66.0	*	67.9
09	Efamol	3	3	2	2	1	2	2	2	2	2	*	3	3	4	3	*	62.1	60.0	59.5	59.8
14	Efamol	3	3	3	3	3	1	1	1	1	1	*	3	3	4	3	85.8	86.2	85.0	*	85.3
16	Efamol	3	3	2	2	2	2	2	2	2	2	*	3	2	2	2	54.2	*	54.5	55.6	54.9
17	Efamol	3	3	3	2	2	2	2	3	3	2	*	3	4	3	2	67.1	67.7	67.3	*	68.4
18	Efamol	1	2	2	*	2	2	3	3	*	3	*	2	2	*	2	*	71.6	72.2	*	69.7
20	Efamol	3	*	*	*	*	3	*	*	*	*	*	*	*	*	*	53.7	*	*	*	*
21	Efamol	3	2	3	1	2	2	2	3	2	2	*	3	4	3	3	55.0	55.4	54.7	54.3	*
24	Efamol	2	3	1	1	1	3	3	2	2	1	*	3	2	2	1	57.4	57.8	56.9	*	55.7
25	Efamol	3	1	*	*	*	3	2	*	*	*	*	2	*	*	*	90.3	90.5	*	*	*
28	Efamol	2	*	2	*	*	3	*	2	*	*	*	*	2	*	*	49.0	*	57.4	*	*
30	Efamol	3	2	*	*	*	1	1	*	*	*	*	2	*	*	*	81.7	81.1	*	*	*
33	Efamol	2	3	2	2	1	3	3	2	2	2	*	3	2	2	2	74.0	*	79.7	80.6	78.1
34	Efamol	2	3	2	2	1	2	2	2	2	1	*	3	3	3	3	61.6	60.7	60.8	60.4	60.4
36	Efamol	2	3	2	2	2	3	3	3	3	3	*	3	3	3	3	54.0	55.2	54.8	55.5	55.8
38	Efamol	2	1	2	1	1	2	1	1	1	1	*	1	1	1	1	48.3	47.5	47.8	46.7	48.5
39	Efamol	2	1	2	2	2	2	2	2	2	2	*	2	2	2	3	59.9	61.1	61.2	*	61.9
42	Efamol	3	3	2	2	2	2	2	2	1	1	*	3	3	3	3	67.7	*	67.2	67.2	68.3
44	Efamol	2	2	1	1	1	2	2	2	1	1	*	3	2	1	2	59.1	60.4	60.0	*	60.5
45	Efamol	3	3	3	2	2	2	2	2	2	2	*	3	2	2	2	47.7	49.2	49.4	51.2	51.0
46	Efamol	3	3	3	3	3	1	1	1	1	1	*	3	4	2	3	58.8	59.2	54.9	58.2	58.1
48	Efamol	2	2	2	1	1	2	2	2	2	2	*	3	3	2	2	53.0	53.0	52.0	53.8	53.2
02	Placebo	2	1	1	2	*	1	1	1	1	*	*	1	1	4	*	*	55.9	*	55.7	*
04	Placebo	2	2	2	*	3	3	2	2	*	3	*	3	2	*	3	*	*	51.4	*	49.4
07	Placebo	3	0	3	2	1	2	0	2	2	1	*	0	2	2	1	65.8	*	66.4	60.8	67.9
08	Placebo	3	2	*	2	1	3	2	*	2	2	*	3	*	2	1	*	*	*	*	66.1
10	Placebo	2	2	1	2	1	1	1	1	1	1	*	3	3	2	2	75.7	77.1	*	*	76.8
11	Placebo	2	3	2	2	2	3	3	2	2	3	*	3	2	2	2	49.4	49.8	49.3	49.3	48.8
15	Placebo	3	1	1	*	1	2	2	1	*	2	*	3	1	*	2	*	51.4	50.8	*	50.6
19	Placebo	2	3	2	3	2	2	3	2	2	2	*	3	2	3	3	62.5	63.5	64.7	63.2	65.0
22	Placebo	3	3	2	1	1	3	3	2	2	1	*	3	2	2	1	81.4	80.5	80.9	80.9	82.5
23	Placebo	2	2	3	*	*	2	2	2	*	*	*	3	3	*	*	47.2	50.6	49.3	*	*
26	Placebo	3	3	3	*	3	2	2	3	*	2	*	3	3	*	3	58.6	58.6	59.0	*	60.0
27	Placebo	2	2	2	3	2	2	2	2	3	2	*	3	2	3	2	59.1	59.6	60.0	*	58.8
29	Placebo	2	2	*	2	3	2	2	*	2	2	*	3	*	3	3	56.8	56.1	*	57.4	58.5
31	Placebo	3	2	*	1	1	3	2	*	1	2	*	3	*	2	1	60.2	61.2	*	61.4	59.9
32	Placebo	3	3	3	1	1	2	2	2	2	2	*	3	2	2	1	56.6	56.6	57.1	55.0	54.7
35	Placebo	3	3	2	1	1	3	3	3	2	1	*	3	3	2	1	59.9	60.2	61.2	61.0	60.5
37	Placebo	3	2	2	3	2	2	2	2	2	2	*	3	3	4	3	60.3	59.6	*	59.2	59.8
40	Placebo	3	2	2	2	3	3	2	2	2	3	*	3	3	2	3	56.1	55.7	*	56.7	56.7
41	Placebo	2	2	2	2	1	2	2	3	2	2	*	2	3	2	2	66.5	66.0	67.0	67.3	68.4
43	Placebo	2	2	2	2	2	2	1	1	1	1	*	3	4	2	4	65.8	65.8	65.8	*	68.2
47	Placebo	2	2	2	1	2	1	1	1	1	1	*	2	4	2	3	65.0	65.9	66.0	66.5	65.0

Assessment Code 0 = Not examined 1 = Asymptomatic 2 = Some improvement
3 = No improvement 4 = Worse
Menstrual Cycle 1 = Regular 2 = Irregular 3 = None

EPO Trial - Raw Data - Pain Chart Scores

Placebo -> EPO Days of Pain

Trial No	Pretrial	Month 1	Month 2	Month 3	Month 4
4	-	-	-	-	-
7	-	-	-	-	-
8	20	25	14	13	3
10	31	28	29	28	22
11	27	-	-	-	-
15	18	-	-	-	-
19	20	18	23	19	22
22	25	16	17	8	8
26	22	20	23	-	-
27	27	-	-	-	-
29	18	20	22	21	7
31	-	-	-	-	-
32	29	29	26	10	17
35	21	25	23	17	8
37	24	21	26	28	29
40	24	31	31	29	30
41	14	12	14	11	13
43	18	20	27	17	13
47	22	11	29	15	20

EPO Trial - Raw Data - Pain Chart Scores

EPO -> EPO Days of Pain

Trial No	Pretrial	Month 1	Month 2	Month 3	Month 4
1	30	23	15	15	6
3	23	19	17	9	6
6	20	22	20	21	11
9	27	18	27	18	10
14	29	31	30	-	-
16	24	19	17	17	13
17	20	24	25	21	17
18	20	6	9	8	9
21	15	18	11	11	10
24	17	20	12	10	1
33	-	-	-	-	-
34	-	-	-	-	-
36	28	16	29	12	12
38	-	-	-	-	-
39	19	8	11	18	22
42	-	-	-	-	-
44	19	22	18	9	6
45	23	21	17	17	10
46	19	15	30	17	20
48	20	24	20	19	8